

# **EFFECT OF FERTILIZER APPLICATION AND IRON REDUCING BACTERIA ON THE RELEASE OF ARSENIC IN THE AQUIFERS OF WEST BENGAL**

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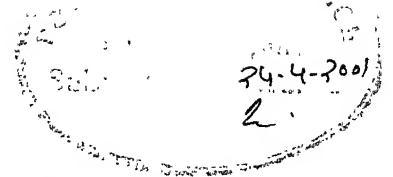
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# CERTIFICATE



It is certified that the work contained in the thesis entitled "*Effect of Fertilizer Application and Iron Reducing Bacteria on the Release of Arsenic in the Aquifers of West Bengal*", by "Mr. Souvik Majumdar" has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

A handwritten signature in black ink, reading "Saumyen Guha".

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And finally, mamoni, you know just who you are.

# ABSTRACT

Arsenic contamination in West Bengal spreads over 8 districts, 58 blocks and 830 villages, thus affecting a total area and population of 38,000 km<sup>2</sup> and 38 million, respectively. In Bangladesh, the scale of the problem is even higher, with 54 districts out of 64 having arsenic concentrations in groundwater above 0.01 mg/L and 47 districts above 0.05 mg/L. The source of arsenic in the sediments of the Ganges delta aquifer has been attributed primarily to geological origins. Although, at present there is no single hypothesis that can satisfactorily explain the release of this sorbed arsenic in the groundwater, a consensus has been reached by the scientific community about a combined biogeochemical process to be the probable reason. In this study, various hypotheses and especially, the effect of iron-reducing bacteria and fertilizer were studied in fixed bed column experiments.

Soils from Ghetughachi, an arsenic affected region in West Bengal, were loaded with arsenic artificially and was used as the porous media for the column experiments. The effects on arsenic leaching by groundwater in aerobic conditions, by fertilizer in both aerobic and anaerobic conditions, by only bacteria, and by bacteria in the presence of added fertilizer were evaluated by column experiments. The bacteria in the presence of added fertilizer mobilized maximum amount of arsenic from the soils. The average concentrations released in the groundwater being 0.92 mg/L in the presence of bacteria and fertilizer, and 0.7 mg/L in the presence of bacteria but without any addition of fertilizer. Whereas the maximum concentration of arsenic released in the abiotic experiments never exceeded 0.2 mg/L. It was concluded that the iron-reducing bacteria could enhance the release of arsenic from the soil to a large extent. Addition of fertilizer can enhance the leaching of arsenic by the bacteria even further. The release of arsenic due to phosphate and aeration are much smaller compared to the microbiological action.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Arsenic Toxicity in the Ganges Delta

Arsenic toxicity in groundwater in southern West Bengal (India), and southern and eastern parts of Bangladesh, is an alarming environmental problem that was first recognized about two decades ago. The presence of arsenic in the groundwater at concentrations that exceed the permissible limit of 0.01 mg/L (WHO 1993, provisional specification < 0.05 mg/L) for human consumption was first recorded in West Bengal in 1978, and the first case of arsenic poisoning was diagnosed in 1983. Within a span of twenty years, over 3 to 4 million people in six districts in southern West Bengal, which is located to the east of the Bhagirathi River (Fig. 1.1), were found to be affected by arsenic poisoning in groundwater (Acharyya *et. al.*, 2000).

In 1987, Chakraborty and Saha reported arsenical dermatosis in 5 districts of West Bengal and found 197 patients of arsenical dermatosis in 48 families. During the period January 1989 to January 1996 the School of Environmental Studies (SOES), Jadavpur University, Kolkatta, conducted a survey in 7 districts of West Bengal, *viz.*, Malda, Murshidabad, Bardhman, Nadia, Hoogli, 24-Parganas (North) and 24-Parganas (South). They reported the presence of arsenic in groundwater more than the permissible limit (0.05 mg/L), in 50 blocks of these seven districts (560 villages including many municipal areas) covering an area of 37,493 km<sup>2</sup> having about 34 million people. According to their report 1.0 million people are drinking arsenic contaminated water and more than 200,000 people have arsenical skin lesions. They analysed about 20,000 tube wells and found that 45% of these tube wells have arsenic content above 0.05 mg/L. They found

the average concentration of arsenic in contaminated water to be about 0.20 mg/L; the maximum concentration of arsenic found out was 3.7 mg/L (Mandal *et. al.*, 1996). According to the report some blocks like, Domkal of Murshidabad, Kalichalk in Malda are seriously affected, and some blocks not so much affected as Baruipur and Sonarpur in South 24-Parganas, while some were moderately affected as Basirhat I, Deganga of North 24-Parganas (Mandal *et. al.*, 1997). In their report they also suggested the possibility of the neighbouring two districts Howrah and Medinipur being affected. Finally in 1997 SOCE reported that 8 districts, 58 blocks and 830 villages are affected. The total area and population of these districts are 38,000 km<sup>2</sup> and 38 million respectively (Dhar *et. al.*, 1997).

In Bangladesh, the arsenic contamination of the groundwater was first detected in 1993. SOES in 1997 reported that they suspected 34 districts of the total 64 districts to be arsenic affected. The reasoning was that the arsenic-affected areas of West Bengal lie in the Younger Deltaic Deposition (YDD), and the same sediment extends eastwards towards Bangladesh, covering the approximate area of 34 districts out of a total 64 districts (Dhar *et. al.*, 1997). In 1988 they reported that in 41 out of 64 districts arsenic in groundwater was above 0.05 mg/L.

In a latest report (April 2000), by SOES and Dhaka Community Hospital (DCH) it has been stated that out of 64 districts in Bangladesh in 54 districts arsenic in groundwater is above 0.01 mg/L and in 47 districts above 0.05 mg/L. A total of 918 villages have been identified where groundwater has arsenic concentrations above 0.05 mg/l. Thirty-two districts were surveyed for arsenic patients and in 30 districts patients with arsenical skin-lesions have been identified. Also it has been added in the report that the people exposed to arsenic concentrations above 0.01 mg/L and 0.05 mg/L from 43 districts are 51 million and 25 million respectively.

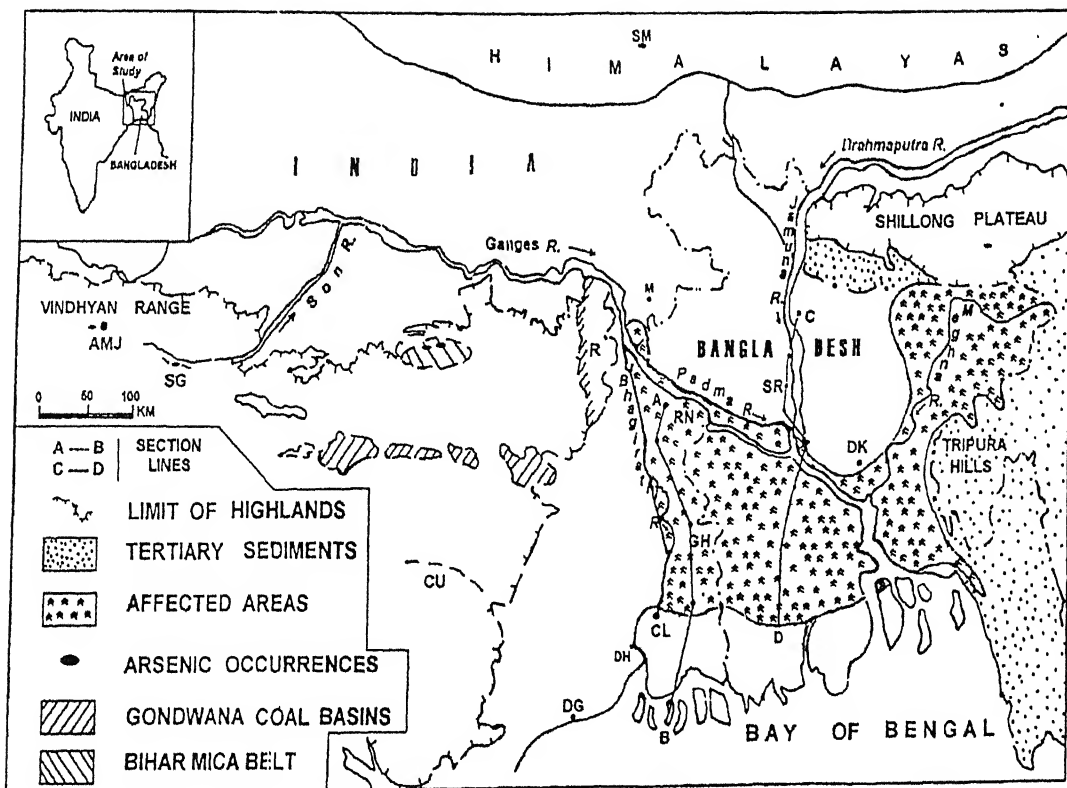


Figure 1.1 Map of arsenic-affected areas in parts of the Bengal basin and potential sources. *AMJ* Amjhore pyrite mine, *CL* Calcutta, *CU* Copper belt Bihar, *DH* Diamond Harbour, *DG* Digha, *DK* Dhaka, *GH* Ghetugachi, *M* Malda, *R* Rajmahal hills, *RN* Rainagar, *SG* Son Valley gold belt, *SM* Samthar, *SR* Siraganj (Acharyya *et. al.*, 2000).

## 1.2 Arsenic in the Ganges Delta Aquifer

The source of arsenic in the Ganges delta aquifer may be either anthropogenic or natural. Anthropogenic emissions of arsenic arise from fuel consumption, mining, smelting of ores and the use of arsenic in fertilizers and pesticides. For instance, acidic mine waters could have dissolved arsenic content as high as 250 mg/L (Aswathanarayana, 1997). These possibilities do exist in West Bengal, and high arsenic content could be an artifact to mining and smelting activities during the last couple of centuries. But there is no

reason to believe that these emission rates could have been higher in the past than at present. This could imply that sediments at the depth from which drinking water is being presently drawn, could not have acquired its arsenic content from man-made sources. Also, if the present and past emission rates are assumed to remain same, surface soil should have at least as much arsenic contamination as in the lower region of the aquifer. However, arsenic contamination in the Ganges delta is seldom reported at depths less than 15 m. It is, therefore, quite justifiable to attribute the arsenic content in the groundwater of the Ganges delta to natural sources (Aswathanarayana, 1997).

The arsenic-affected area, as initially recognized in West Bengal and Bangladesh, is mostly confined to the lower flood-delta plains of the Ganges River, downstream of the Rajmahal Hills (Fig. 1.1). The Gangetic alluvial tract upstream of the Rajmahal Hills, in the states of Bihar and Uttar Pradesh (UP), in India, is generally free of arsenic toxicity. However, the adjacent part of the Ganges delta in Bangladesh is equally affected. It was further recognized that arsenic toxicity in Bangladesh extends also to the Sylhet basin and flood-delta plains of the River Meghna in eastern and southern Bangladesh (Fig. 1.1).

The wide expanse and regional extent of the arsenic-toxic zone in the Ganges delta in West Bengal and Bangladesh, as well as, those in the Meghna River floodplain and in the Sylhet basin, in Bangladesh (Fig. 1.1), which all have different catchment areas, indicates multiple natural sources of arsenic contamination. In contrast, the Brahmaputra-Jamuna, and old Brahmaputra floodplains in Bangladesh, are so far unaffected, although these areas are subjected to similar levels of groundwater extraction compared with other areas that are found to be arsenic toxic (Acharyya *et. al.*, 2000).

Likely sources for arsenic in the Ganges delta may include the following (Acharyya *et. al.*, 2000):

- (i) the Gondwana coal seams in Rajmahal basin contain up to 200 ppm of arsenic,
- (ii) the arsenic mineral lollingite and pyrite, which occur sporadically in association of pegmatites in the mica-belt of Bihar, have an arsenic content in mineralized rocks that ranges from 0.12-0.08% (Bhattacharya, 1972),
- (iii) pyrite bearing shale from the Proterozoic Vindhyan range, with its Amjhore mine, contains 0.26% As (Das, 1977),
- (iv) the gold belt of the Son Valley has an arsenic content in the bedrock that locally reaches 2.8% to 1000 ppm (Mishra *et. al.*, 1996),
- (v) isolated outcrops of sulphides contain up to 0.8% of arsenic in the Darjeeling Himalayas, and
- (vi) in outcrops in the upper reaches in the Ganges River system.

The copper belt of Bihar, and the Damodar valley Gondwana coal basins contain local concentrations of arsenic in pockets that are drained by rivers that flow far to the south of the Ganges tributary system, and thus these cannot be potential sources as postulated by some (Nickson *et. al.*, 1999). Further, the Damodar alluvial fan that flanks the arsenic-affected Bhagirathi delta is free of arsenic problems.

The ongoing mineralogical studies indicate that arsenic-rich pyrite and separate arsenic minerals are absent or rare in the sediments of the Ganges delta of West Bengal. However, arsenic appears to be adsorbed to iron-hydroxide-coated sand grains and to clay minerals (Acharyya *et. al.*, 1999). The rare presence of authigenic pyrite has been recognized from the Ghetughachi area and is also reported from Bangladesh (Nickson *et. al.*, 1998). In the Ghetughachi area, the presence of arsenic was noted in a rare case within iron hydroxide- and sulphide-coatings on heavy mineral illeminite. Sparse occurrence of these arsenic-bearing minerals cannot explain the large-scale arsenic contamination in the deltaic plane. Thus, it is likely that arsenic was co-precipitated with

or scavenged by iron (III) and manganese (IV) in the sedimentary environment. The presence of arsenopyrite or the pyrite-bearing layers or zones in the aquifer, as has been postulated by some (Das *et. al.*, 1996; Mallick and Rajagopal 1996) is doubtful. Instead it is likely that decomposition of pyrite and arsenic bearing sediments in the source area released arsenic in the soluble form, and this was sorbed by secondary iron oxyhydroxide. Arsenic in solution possibly became more easily entrapped in the fine-grained organic-matter-rich sediments that were preferentially deposited under low energy conditions in the Ganges delta (Acharyya *et. al.*, 2000).

### **1.3 Mobilization of Arsenic in Groundwater**

Several models have been proposed to explain the leaching of arsenic from aquifer material to the groundwater of the Ganges delta. These include

- (a) oxidation of arsenical pyrite (Mallick and Rajagopal 1996; Mandal *et. al.*, 1998; Roychowdhury *et. al.*, 1999)
- (b) competitive anion exchange of sorbed arsenic with phosphate from fertilizer (Acharyya *et. al.*, 1999, 2000), and
- (c) reductive dissolution of FeOOH and release of the sorbed arsenic in to the groundwater (Bhattacharya *et. al.*, 1997; Nickson *et. al.*, 1998, 2000).

These theories are discussed in the following few pages.

#### ***Oxidation of Arsenical Pyrite***

This theory suggests that the main source of arsenic in this region is an arsenopyrite layer in the aquifer (Das *et. al.*, 1996; Mallick and Rajagopal 1996). Due to extensive pumping and subsequent water table draw down, the dissolved oxygen content has increased in the aquifer and exposed the arsenopyrite layer near the cone of depression. Oxidation of these sulphides has resulted in the release of arsenic in the groundwater. It



has also been suggested that the water entering through the strainer of a tubewell to maintain discharge, acts like a stirrer for the entire length of the aquifer in contact with the well. If the source of arsenic is located somewhere in the vicinity of the well, it can get mixed up with the entire volume of water.

However this theory suffers from the drawback, that the presence of such an arsenopyrite or pyrite-bearing layers or zones in the aquifer, is doubtful (Acharyya *et. al.*, 1999, 2000). Also according to McArthur *et. al.* (2000), measured sulphur concentrations in the aquifer sediments represent both pyrite and organic sulphur but allow upper limit to be placed on pyrite abundance of 0.3% (Nickson *et. al.*, 2000), 0.02% (AAN, 1999), 0.1% (J. M. McArthur unpublished) and 0.06% (DPHE, 1999). According to them the presence of pyrite shows that it has not been oxidized and that it is a sink, not a source of, arsenic in Bangladesh groundwater. If pyrite were oxidized then its arsenic would have been sorbed to the resulting FeOOH (Mok and Wai, 1994; Savage *et. al.*, 2000), rather than released to the groundwater. Furthermore, they point out that, Bangladesh groundwaters, which are anoxic in nature, would have contained iron and sulphate in molar ratio of 0.5, if pyrite oxidation released arsenic; but they were found to be mutually exclusive in solution (DPHE, 2000), as are arsenic and sulphate, *i.e.* arsenic concentrations above 50 µg/L were found only where sulphate concentrations were less than 30 mg/L (DPHE, 1999, 2000). And finally, arsenic pollution is uncommon in hand-dug wells (DPHE, 1999), which are shallowest, and most exposed to atmospheric oxygen and so should have been most polluted were arsenic derived from pyrite oxidation. In this study, this option was kept open and was studied further.

#### ***Competitive Anion Exchange of Sorbed Arsenic with Phosphate from Fertilizer***

Recently, Acharyya *et. al.* (1999, 2000), has suggested that phosphate may play a major role in arsenic contamination. During the past thirty years, ground water has been

increasingly used for irrigation and the use of phosphate fertilizers has increased three folds. This widespread withdrawal of groundwater may have mobilized phosphate from fertilizers and from decay of natural organic material in the shallow aquifers. Phosphate in groundwater can leach out arsenic from the soil (Dubey, 2000). In the present research, this option was also explored into.

#### ***Reductive Dissolution of FeOOH and Release of the Sorbed Arsenic to groundwater***

In this hypothesis, it is suggested that arsenic is released by the reductive dissolution of iron oxyhydroxides (FeOOH) (Bhattacharya *et. al.*, 1997; Nickson *et. al.*, 1998, 2000). In the groundwater of a few wells in Bangladesh, Nickson *et. al.* (1998) observed that arsenic correlates with dissolved iron and with bicarbonate concentrations above 300 mg/L; the latter is a by-product of iron oxyhydroxide reduction. This of course indicates the presence of a reducing environment at the region of arsenic contamination. However, if it is agreed that arsenic contamination is a recent catastrophe, then this theory cannot explain the increase of arsenic concentrations in groundwater in the past three decades.

Recently, another theory is surfacing that takes into account the biogeochemical effect. Both Nickson *et. al.* (2000) and Acharyya *et. al.* (2000) have pointed towards the role of micro-organisms in the leaching of arsenic from the sediments. Nickson *et. al.* (2000) has suggested the reduction of Fe is driven by microbial metabolism of sedimentary organic matter. Whereas, Acharyya *et. al.* (2000) has pointed that a combined microbiological and chemical process might better explain the recent release of arsenic in groundwater.

Batch experiments conducted on arsenic contaminated soil samples showed that iron reducing bacteria can enhance the leaching of arsenic in the groundwater, provided nutrient is not a constraint (Ghosh, 2000). The presence of such bacteria, in the arsenic containing aquifer sediment was also confirmed. When external substrates and nutrients

were supplied the release of arsenic increased. Also, it was shown that external phosphate is capable of enhancing the release of arsenic from the soil (Ghosh, 2000).

The enhanced leaching in presence of iron reducing bacteria can be very well appreciated. In anoxic conditions Fe(III) can act as an electron source. In that case iron oxyhydroxide will be reduced. This will in effect release the arsenic in the groundwater that exists as a dispersed phase, *e.g.* as a coating, on sedimentary grains.

## CHAPTER 2

### OBJECTIVE OF THE STUDY

From the literature review in the previous chapter, it seems that the arsenic contamination is most probably associated with reductive dissolution of iron oxyhydroxides in presence of iron reducing bacteria (Ghosh, 2000). But to generalize this statement and to say that iron-reducing bacteria are equally active in leaching out arsenic in the Ganges delta aquifer, will be an overestimate. The experiments that were performed were all batch experiments in closed containers where there was absolutely no flow. But groundwater in the Ganges delta aquifer is characterized by flow of water across the aquifer. Although, under normal conditions the groundwater movement is very slow in the arsenic-affected tract in West Bengal owing to its flat nature (average slope is 10 cm/km). The groundwater movement is of the order of 500 cm per year in horizontal direction and still less as far as vertical movement is concerned (Mallick and Rajagopal, 1996). The result is that groundwater in the Ganges delta aquifer stays in contact with the arsenic containing sediments for a particular period of time. However, this contact time is reduced to a large extent in the vicinity of the wells. What remains to be seen is that whether iron-reducing bacteria is effective in leaching out arsenic from the sediments under flow conditions through a fixed bed.

Keeping all three hypotheses of arsenic release (section 1.3) in mind column experiments were conducted with soils from the West Bengal aquifer to examine the following:

- (i) arsenic leaching by groundwater in oxic condition,
- (ii) the effect of fertilizer on the leaching of arsenic in the groundwater in oxic condition,

- (iii) the effect of fertilizer on the leaching of arsenic in the groundwater in anoxic condition,
- (iv) the effect of iron-reducing bacteria on arsenic leaching, and
- (v) the effect fertilizer on the arsenic leaching mediated by iron-reducing bacteria.

# CHAPTER 3

## MATERIALS AND METHODS

### 3.1 Soil Sample

Sand samples used belonged to an arsenic containing aquifer in 'Ghetughachi', North 24-Parganas, West Bengal. The Geological Survey of India (GSI), Kolkata, collected these samples from different depths of two boreholes, BH-1 (19 – 60 m) and BH-4 (34.1 – 46.55 m). These samples were collected in polythene bags and sealed containers; stored and preserved in the laboratory in a refrigerator at  $\sim 4^{\circ}\text{C}$ .

### 3.2 Analytical Methods

All reagents were prepared using fresh distilled water prepared in the laboratory. Chemicals used were all of analytical reagent grade and were used as received.

#### 3.2.1 Analysis of Arsenite, Arsenate and Phosphate by the Molybdenum Blue Method

The molybdenum blue method (Johnson and Pilson, 1972) was used with a slight modification for the determination of arsenite, arsenate and phosphate. This method allows for the simultaneous colourimetric determination of As(III), As(V) and  $\text{PO}_4^{3-}$ ; and depends on the principle that both arsenate and phosphate forms a blue complex with molybdate while arsenite does not. The reduction of arsenate to arsenite eliminates its absorbance in phosphate measurements, whereas oxidation of arsenite to arsenate adds to the absorbance values obtained for an unoxidised portion of the sample. Thus, under appropriate conditions the absorbance of a reduced sample is due to that of phosphate alone; the difference in absorbance between an untreated (*i.e.* unreduced) and reduced

sample can be taken as a measure of the arsenate present; and finally, the difference in the absorbance between the oxidised and untreated sample is that due to arsenite alone. Thus, if three aliquots of a sample are taken, and one “oxidised”, one “reduced” and the third part left “untreated”, then it can be said that

Abs. due to  $\text{PO}_4^{3-}$  = Abs. of the “reduced” aliquot

Abs. due to As(V) = Abs. of “untreated” sample – Abs. of “reduced” aliquot

Abs. due to As(III) = Abs. of “oxidised” aliquot – Abs. of “untreated” aliquot

Details of this method are given in the Appendix.

The standard curves of arsenic and phosphate obtained by this method are given in Fig. 3.1a,b and Fig. 3.2a,b. Absorbances were measured at 865 nm using path-lengths of both 10 mm and 40 mm

### 3.2.2 Analysis of Arsenic by SDDC Method

The total arsenic was also analysed using the Silver Diethyl Dithiocarbamate (SDDC) method as described in the Standards Methods (1985) with a slight modification. In this method, inorganic arsenic is reduced to arsine,  $\text{AsH}_3$ , in the presence of nascent hydrogen in a Gutzeit generator. The arsine is then passed through a scrubber containing SDDC solution in pyridine (1.0 g of SDDC in 100 mL of pyridine). Two modifications were introduced here; first, by using 3 mL of SDDC solution instead of 4 mL and second, by extending the reaction period to one hour from half an hour as suggested by the Standard Methods (1985). In the absorber arsine reacts with the silver salt to form a red coloured compound suitable for spectrophotometric measurement. The absorbance was measured at 510 nm, using a path-length of 10 mm. A standard curve was prepared and is shown in Fig. 3.3.

In case of soil samples, a slurry was made with an appropriate weight of dry sample in 35 mL distilled water taken in a clean generator bottle. The rest of the method was very

similar to that described above for aqueous samples except that the generator bottle was shaken by hand during the entire arsine generation period

### 3.2.3 Analysis of Iron by the 1,10-phenanthroline Method

Iron was analysed by the 1,10-phenanthroline method as described in the Standard Methods (1985). For the determination of total iron, 50 mL of the sample solution was taken and the  $\text{Fe}^{3+}$  present was reduced to  $\text{Fe}^{2+}$  by 1 mL hydroxylamine ( $\text{NH}_4\text{C}_2\text{H}_4\text{H}_2\text{O}$ ) in the presence of 2 mL concentrated hydrochloric acid. The solution was boiled until its volume was reduced to 15 – 20 mL. After cooling, 10 mL of acetate buffer ( $\text{NH}_4\text{C}_2\text{H}_2\text{O}$  in glacial acetic acid) and 4 mL of 1,10-phenanthroline solution was added and the volume made up to 50 mL. Absorbance was noted after 10 – 15 minutes at 510 nm using a 10 mm cell. A standard curve for iron was made using the above procedure. The standard curve is shown in Fig. 3.4.

For  $\text{Fe}^{2+}$ , 50 mL portion of a sample was taken in a 100 mL volumetric flask and acidified with 1 mL concentrated hydrochloric acid. Twenty mL of 1,10-phenanthroline solution and 10 mL of ammonium acetate buffer solution were added immediately in the flask and the volume made up to the 100 mL mark. The colour intensity was then measured within 15 minutes at a wavelength of 510 nm using a 10 mm cell.



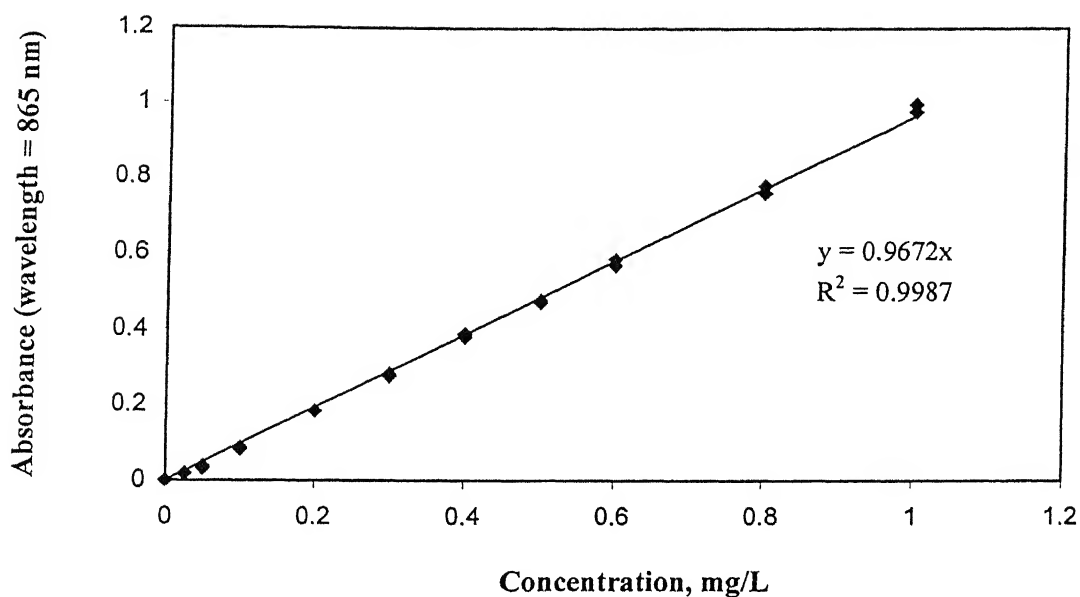


Figure 3.1 (a) Standard Curve for Arsenic using the Molybdenum Blue Method (path-length = 40 mm).

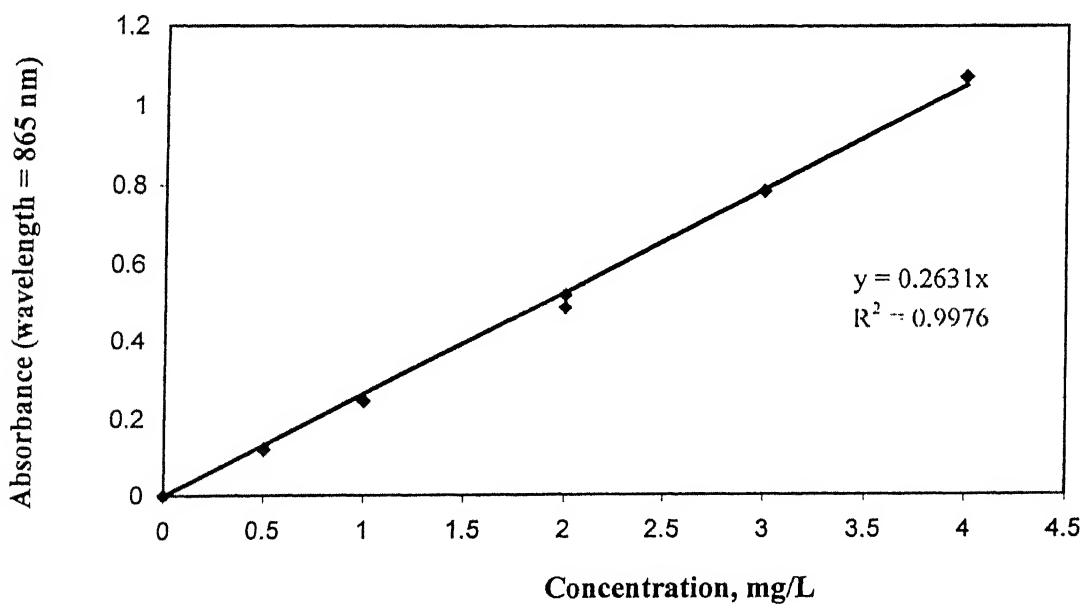


Figure 3.1 (a) Standard Curve for Arsenic using the Molybdenum Blue Method (path-length = 10 mm).

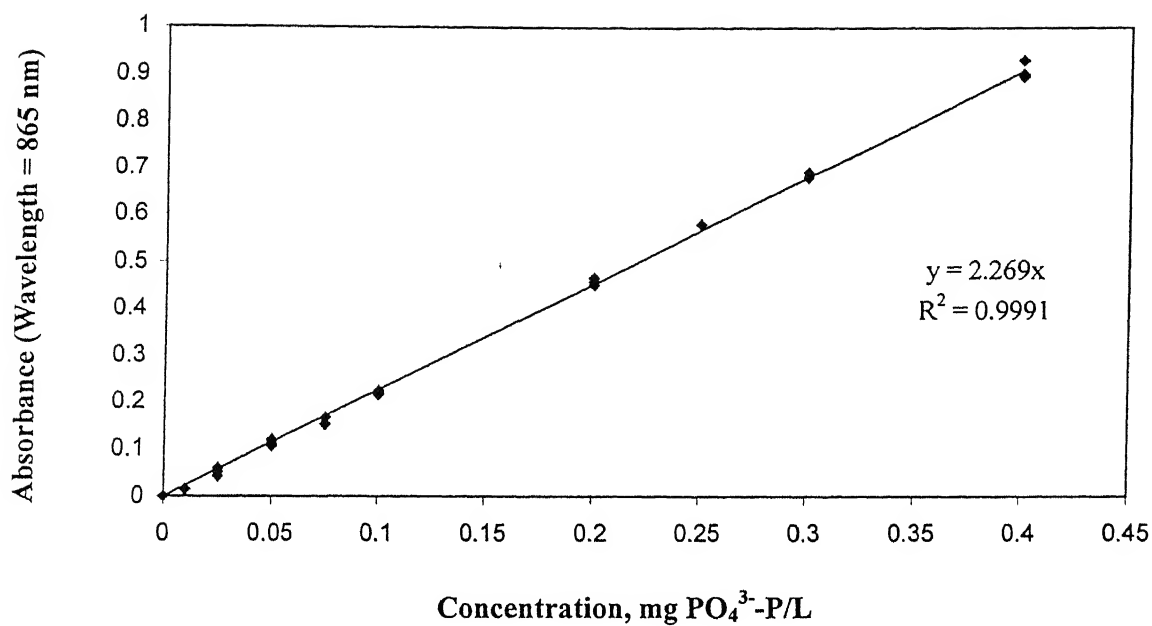


Figure 3.2 (a) Standard Curve for Phosphate using the Molybdenum Blue Method (Light Path = 40 mm).

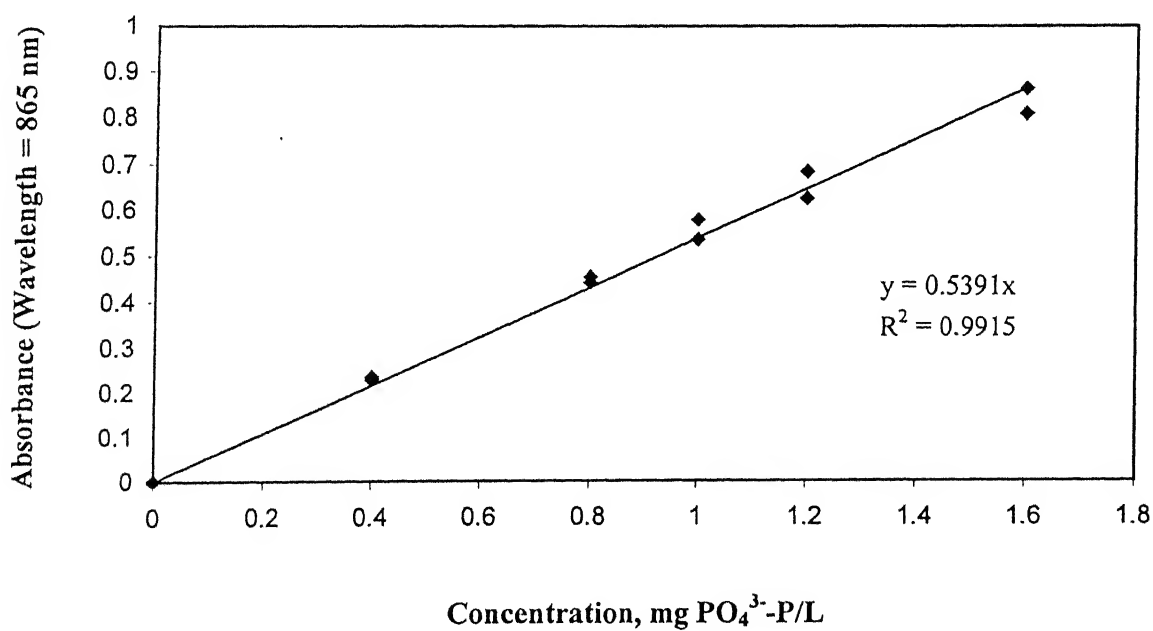


Figure 3.2 (b) Standard Curve for Phosphate using the Molybdenum Blue Method (Light Path = 10 mm).

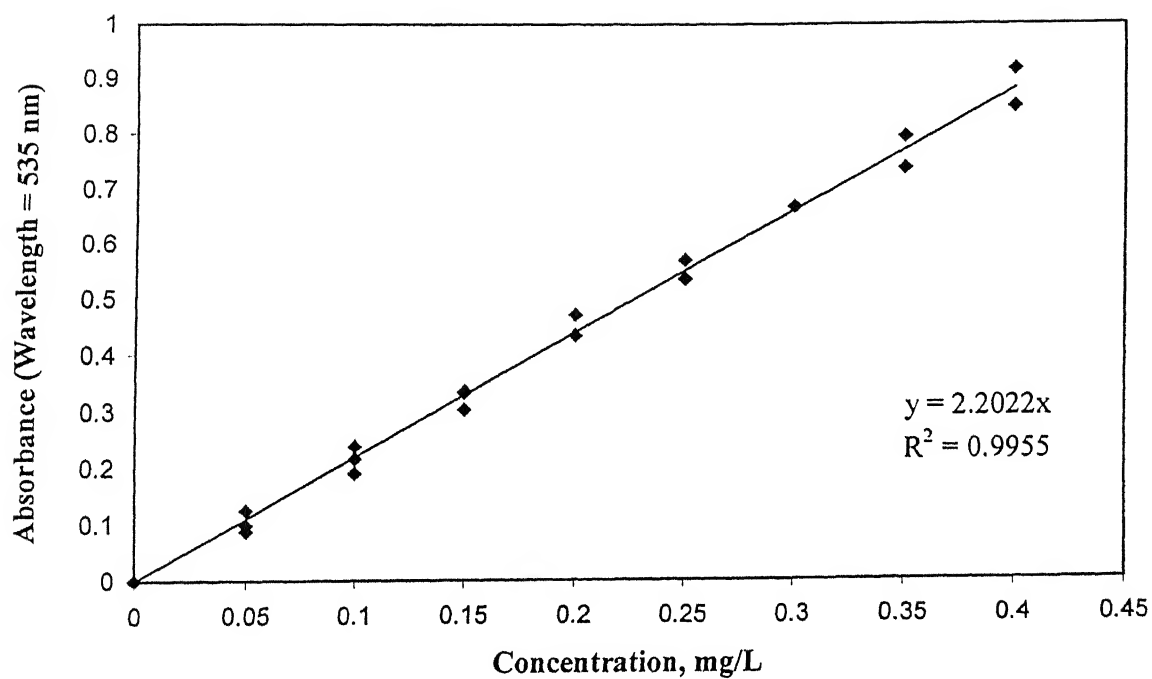


Figure 3.3 Standard Curve for Arsenic using the SDDC Method (Light Path = 10 mm).

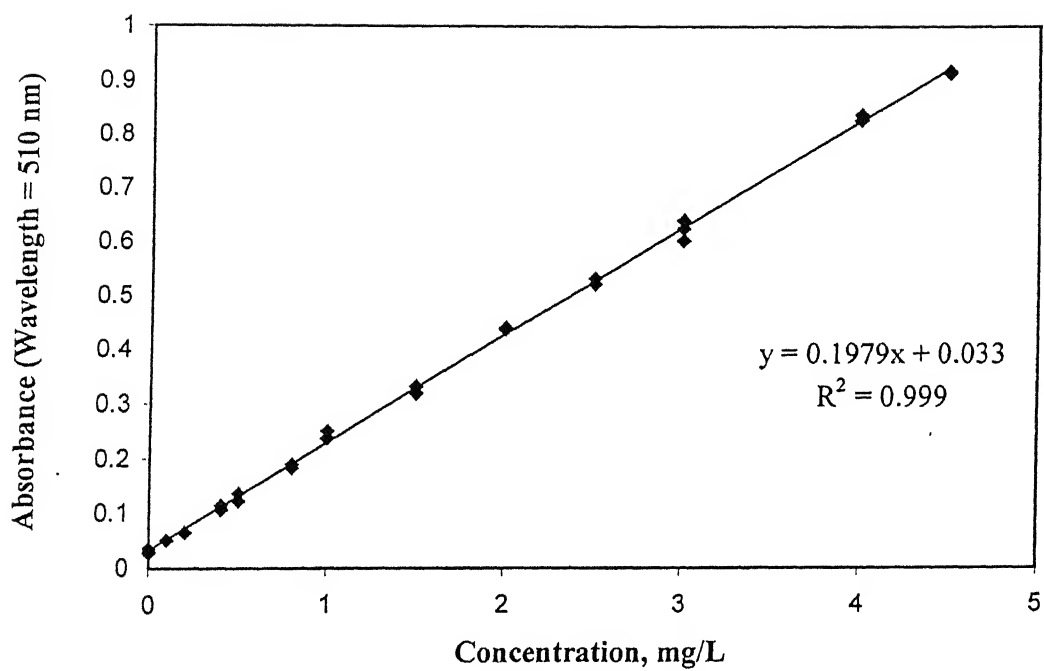


Figure 3.4 Standard Curve for Iron using the 1,10-phenanthroline Method (Light Path = 10 mm).

### 3.3 Experimental Methodology

#### 3.3.1 Loading of Arsenic on Sand

In the arsenic containing aquifer of Ghetughachi, soil arsenic contents are not uniform. Out of three bore holes (BH-1, BH-3 and BH-4), which are only a few hundred meters apart, BH-3 contains arsenic whereas BH-1 and BH-4 do not, although soil profiles in all three parts of the aquifer are the same. The soil samples collected from BH-3 were used by Ghosh (2000). Sufficient soil samples of BH-3 were not available for performing all the column experiments in the present study. To preserve uniformity, soils from BH-4 were artificially contaminated with arsenic and were used for all the experiments.

A mixture of sodium arsenate,  $\text{Na}_2\text{AsO}_4 \cdot \text{H}_2\text{O}$  (0.35 g) and of sodium arsenite,  $\text{NaAsO}_2$  (0.15 g) were dissolved in 250 mL of distilled water and added to 100 g of dry BH-4 soil kept in a 500 mL stoppered reagent bottle. The bottle was then placed in a rotary shaker rotating at approximately 60 rpm for 48 hours to adsorb arsenite and arsenate on to the soil particles. The bottle was then taken out and sand was allowed to settle for an hour. The supernatant was carefully decanted in a clear beaker and the sand in the bottle dried for 24 hours in an electric oven maintained at  $105^\circ\text{C}$ . The supernatant was centrifuged at 5000 rpm for 30 minutes and the clear solution thus obtained was analysed for arsenite, arsenate and phosphate by the Molybdenum Blue Method. The dried sand was also analysed for total extractable arsenic by the SDDC method as described earlier (section 3.2.2). The weights of sand taken for the analyses were in the range 0.03 to 0.06 g. The arsenic content of the soil was found to be in the range 195.6 to 222.8  $\mu\text{g/g}$  as determined by analysis of 10 samples by the SDDC method.

The concentration of arsenite and arsenate remaining in the solution gave an indirect estimation of arsenic(III) and arsenic(V) adsorbed on to the soil. The SDDC method on the other hand, provided a means for the direct estimation of the total arsenic adsorbed.

Results obtained by the Molybdenum Blue method suggested that 80% of arsenic(V) and 20% of arsenic(III) were adsorbed on to the soil.

### 3.3.2 Culture of Iron-Reducing Bacteria

Anaerobic mixed cultures were isolated from anaerobic sediments collected from a pond in Kanpur. The anaerobic sediments were collected and transported to the laboratory in sealed plastic containers.

Immediately after transportation of the sediments to the laboratory, they were used for the isolation of mixed cultures by the method discussed below.

Five autoclaved culture bottles (approximate volume 190 mL) were taken and to each approximately 5 g of sediment were added. During addition of sediments from each container the upper few centimeters were discarded. This was done in order to assure that only anaerobic sediments were being transferred. If due to some leakage, any oxygen diffused through, it would be only through the upper portion of the sediments.

The enrichment media for iron reducing bacteria (Lovely *et. al.*, 1988) was added to the culture bottles up to the brim and tightly capped. The composition of the media is given in Table 3.1. The enrichment media prepared was autoclaved for 30 minutes, cooled and kept overnight in an incubator maintained at 35 °C before being added to the sediments in the culture bottles.

The five culture bottles were then placed in an incubator maintained at 35 °C (Lovely, *et. al.*, 1988). After seven days of incubation, the bottles were taken out and 50 mL of culture solution were transferred to a series of new bottles. All the bottles were filled with fresh autoclaved enrichment media. The process was repeated to get a considerable number of bacterial culture bottles.

The anaerobic conditions in the culture bottles were maintained using sodium thioglycolate ( $\text{HSCH}_2\text{CO}_2\text{Na}$ ). A concentration of 0.2 g/L of sodium thioglycolate was

found suitable for maintaining anaerobic conditions in the bottles (Ghosh, 2000; Sharma, 2000).

**Table 3.1. Composition of the enrichment media for iron reducing bacteria (Lovely, *et. al.*, 1988).**

Ingredient	Chemical Formula	Amount, g/L in distilled water
Sodium bicarbonate	$\text{NaHCO}_3$	2.5
Calcium chloride	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.1
Potassium chloride	KCl	0.1
Ammonium chloride	$\text{NH}_4\text{Cl}$	1.5
Sodium dihydrogen phosphate	$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.6
Sodium chloride	NaCl	0.1
Magnesium chloride	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.1
Magnesium sulphate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.1
Manganese chloride	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.005
Sodium molybdate	$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.001
Sodium acetate	$\text{CH}_3\text{COONa}$	2.7
Yeast extract	Yeast extract	0.05

Ferric hydroxide was used as the electron acceptor, approximately 2.0 g per culture bottle. This was prepared in the laboratory by adding ammonium hydroxide to an aqueous solution of ferric chloride. The resulting solution was filtered through a Whatman 40 filter paper and the precipitate thus obtained was collected and dried before use.

### *Verifying the presence of iron-reducing bacteria*

Twelve culture bottles were taken for this purpose and among them six were autoclaved to kill the bacteria in the culture, and the other six were kept with live culture. All the bottles were kept in the incubator maintained at 35 °C. Iron(II) concentration, pH and alkalinity were measured in these bottles at 0 hour and at the end of 2, 4, 6, 8 and 10 days of incubation.

In ten days the iron(II) concentration increased by ~ 10 times in the bottle containing bacteria, *i.e.*, from ~ 0.12 mg/L after 0 hours to ~ 1.4 mg/L at the end of the 10<sup>th</sup> day. The same increase in the control bottles was ~ 2 times, from ~ 0.32 mg/L after 0 hours to ~ 0.6 mg/L at the end of the 10<sup>th</sup> day. The bicarbonate alkalinity of the bacterial culture bottles was also found to increase significantly, in comparison to the control bottles. This confirmed the presence of iron reducing bacteria in the culture bottles.

## **3.4 Column Experiments**

To study the effect of fertilizer and iron reducing bacteria in the mobilization of arsenic in the groundwater two types of column experiments were conducted. They are

- (a) aerobic unsaturated column experiments, and
- (b) anaerobic saturated column experiments.

These are described in the subsequent sections.

### **3.4.1 Aerobic Unsaturated Column Experiments**

For these set of experiments a setup similar to that shown in the schematic diagram (Fig. 3.5) was used. The columns used for this purpose were made of glass with a uniform inner radius of approximately 1.8 cm. The lengths of the columns were approximately 25 cm. The columns were packed with soil loaded with arsenic (section 3.3.1) up to a



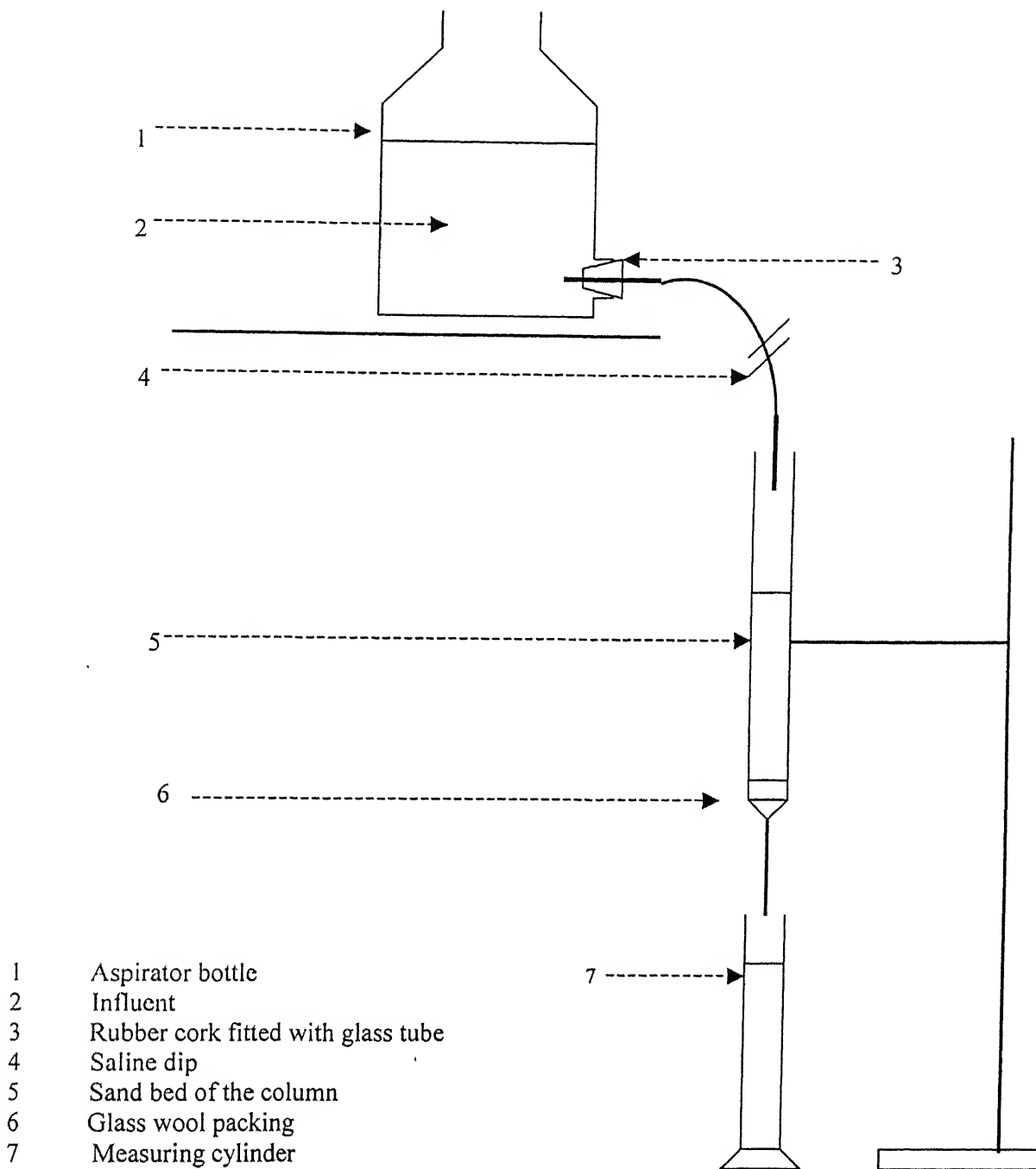


Figure 3.5 Schematic representation of the aerobic experimental setup.

height of 15 cm. To prevent the sand from escaping and to hold the sand bed in place glass wool packing was used at the bottom, the thickness of which was around 0.5 cm. The top of the column was kept open such that the sand bed remained exposed to the atmosphere.

Groundwater was fed to the sand column drop by drop from a 5 L clean aspirator bottle by means of saline dips. The flow rate was so adjusted that the sand bed always remained unsaturated, *i.e.*, no head was allowed to build over the sand bed. The leachates coming out of the column were collected in 250 mL measuring cylinders. Sample collections were made in a manner as to either collect a volume of 250 mL or the volume of leachate that came out in 4 hours, whichever earlier. These were then stored in 250 mL stoppered reagent bottles in a refrigerator for future analysis.

Arsenite, arsenate and phosphate (by the Molybdenum blue method) and total arsenic (by SDDC) were analysed for each sample collected.

Two sets of experiment were conducted with these aerobic columns. They were

- (a) with groundwater only, and
- (b) with groundwater and fertilizer.

#### ***Aerobic unsaturated column experiment with groundwater***

This experiment was conducted with an aim to study the leaching of arsenic from sand using only groundwater of IIT Kanpur. The columns were prepared as described earlier in this section. The experiment was done in triplicate and the columns were run continuously for approximately 59 hours.

The three columns used in this experiment were named as column A, column B and column C. The weights of sand used in preparing the columns and the flow rates are tabulated below:

**Table 3.2** Weights of sand used and flow rates of the three columns used in the aerobic column experiment with groundwater.

Name	Weight of sand used, g	Flow-rate, mL/hr
Column A	46.92	$63.3 \pm 2.5$
Column B	47.02	$65.2 \pm 2.6$
Column C	47.08	$63.2 \pm 4.1$

***Aerobic unsaturated column experiment with fertilizer and groundwater***

This experiment was conducted with an aim to study the effect of fertilizer application on arsenic leaching in groundwater of IIT Kanpur in aerobic conditions. The columns were prepared as described earlier in this section. The experiment was conducted in duplicate and the columns were run continuously for approximately 57 hours.

The fertilizer used was a mixture of urea and DAP (diammonium phosphate) in the ratio 1:2 (w/w). This ratio was chosen in accordance with the ratio of weights of urea and DAP applied in West Bengal which according to information gathered, is 50 kg of urea to 100 kg of DAP per acre.

Approximately 0.15 g of dry fertilizer mixture (0.5 g of urea and 1.0 g of DAP) was sprinkled on top of the sand bed inside the column. Successive additions of fertilizer to the column were made after every 1 L of leachate was collected.

The two columns used in this experiment were named as column A and column B. The weights of sand used in preparing the columns and the flow rates are tabulated below:

**Table 3.3** Weights of sand used and flow rates of the two columns used in the aerobic unsaturated column experiment with fertilizer and groundwater.

Name	Weight of sand used, g	Flow-rate, mL/hr
Column A	46.08	$53.1 \pm 8.3$
Column B	46.13	$52.5 \pm 8.9$

### 3.4.2 Anaerobic Saturated Column Experiments

For these set of experiments a setup similar to that shown in the schematic diagram (Fig. 3.6) was used. The columns used for this purpose were the same as that described elsewhere (section 3.4.1) and the columns prepared likewise. The only difference was that these columns used in the anaerobic experiments were not exposed to the atmosphere. They were tightly stoppered with rubber corks fitted with glass tubes through which the influent supply was maintained. The space above the sand bed and the cork remained completely filled with the influent leaving no headspace.

The mouth of the aspirator bottle was also tightly closed by another rubber cork fitted with two glass tubes of different sizes. The longer of the two tubes was connected to a funnel by means of a rubber tube, and this arrangement became the feeder system of the entire experimental setup. The other smaller tube was connected to a gas balloon containing nitrogen gas. This allowed the headspace in the aspirator bottle to be filled with an atmosphere of nitrogen gas during the experimental run. The influent solution was fed to the column by means of rubber tubes.

The flow-rate in these set of experiments were maintained with the help of rubber tubes fitted with pinch cocks attached to the effluent end of the columns. The experimental setup was closed to the outer atmosphere by placing pinch cocks at appropriate places. All connections were sealed with sticking tapes or adhesives to make them airtight.

Before commencing of the experiment, the influent containing 0.2 g/L of sodium thioglycolate was filled in the aspirator bottle and nitrogen gas bubbled through the system for 30 minutes to drive away oxygen from the bottle. The sodium thioglycolate concentration of 0.2 g/L was found to maintain anaerobic condition in the experimental system for more than 5 days.

The following experiments were conducted with these anaerobic columns

- (i) with groundwater and fertilizer,

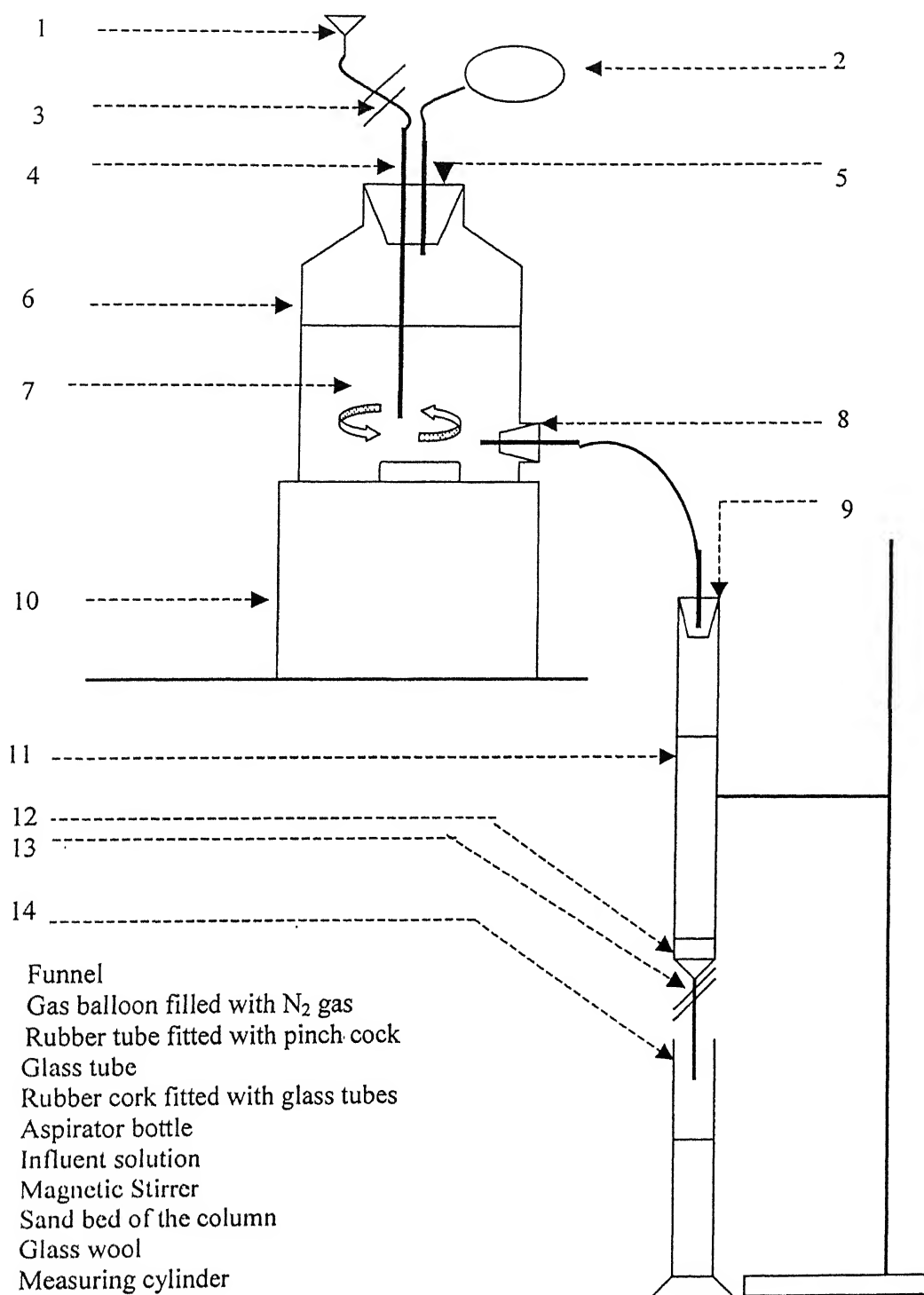


Figure 3.6 Schematic representation of the anaerobic column.

- (ii) with bacteria culture and groundwater, and
- (iii) with bacterial culture, fertilizer and groundwater.

#### ***Anaerobic Saturated Column Experiment with groundwater and fertilizer***

This experiment was conducted with an aim to study the effect of fertilizer application on arsenic leaching in groundwater of IIT Kanpur in anaerobic conditions. The column was prepared as described earlier (in section 3.4.1). The column was run continuously for approximately 57 hours.

The ratio of fertilizer applied was the same as that used in the aerobic column run. The only difference was that the fertilizer was added into the aspirator bottle along with water (0.15 g of fertilizer in 1 L water) that maintained a constant fertilizer concentration in the influent water. DAP obtained commercially, is a very slow dissolving material, and so to keep the DAP in suspension a magnetic stirrer was used. This provided a means of supplying a constant concentration of fertilizer to the column.

. The weight of sand used in preparing the column and the flow rate are tabulated below:

**Table 3.4 Weight of sand used and flow rate of the column used in the anaerobic saturated column experiment with groundwater and fertilizer.**

Name	Weight of sand used, g	Flow-rate, mL/hr
Column C	48.05	62.1 $\pm$ 2.2

#### ***Anaerobic Saturated Column Experiment with groundwater and bacteria***

This experiment was conducted with an aim to study the effect of iron-reducing bacteria on the leaching of arsenic in groundwater of IIT Kanpur. In this case approximately 800 mL of bacterial culture solution was added to 4 L of groundwater containing 0.2 g/L of sodium thioglycolate. To supply a uniform bacterial concentration a magnetic stirrer was used in this case also.

The weight of sand used in preparing the column and the flow rate are tabulated below:

**Table 3.5** Weight of sand used and flow rate of the column used in the anaerobic saturated column experiment with groundwater and bacteria.

Weight of sand used, g	Flow-rate, mL/hr
47.05	$59.1 \pm 3.8$

*Anaerobic Saturated Column Experiment with groundwater, bacteria and fertilizer*

To study the effect of fertilizer on the bacteria mediated arsenic release this column experiments were conducted. This experiment was done in duplicate. This experiment was identical to the previous one except that a fertilizer mixture was added to the influent water.

The two columns used in this experiment were named as column A and column B. The weights of sand used in preparing the columns and the flow rates are tabulated below:

**3.3** Weights of sand used and flow rates of the two columns used in the anaerobic experiment with groundwater, bacteria and fertilizer.

Name	Weight of sand used, g	Flow-rate, mL/hr
Column A	48.6	$61.1 \pm 8.3$
Column B	48.02	$62.5 \pm 8.9$

## CHAPTER 4

# RESULTS AND DISCUSSION

This chapter is divided into the following five sections:

- (a) Arsenic release in groundwater of IIT Kanpur in aerobic condition,
- (b) Arsenic release in groundwater in aerobic and anaerobic condition with fertilizer application,
- (c) Arsenic release in groundwater mediated by iron-reducing bacteria under anaerobic condition,
- (d) Effect of fertilizer application on the release of arsenic mediated by iron-reducing bacteria under anaerobic condition, and
- (e) Discussion of the observed results in the light of various hypotheses proposed for arsenic release in the Ganges delta.

### 4.1 Arsenic Release in groundwater of IIT Kanpur

To study the release of arsenic in the groundwater of IIT Kanpur, aerobic saturated column experiments were conducted. This experiment was conducted in triplicates, and the details are given elsewhere (section 3.4.1). Results are shown in Figures 4.1a-e. The points in the figures indicate 4-hour average concentration of arsenic and phosphate. So the line joining points is the histogram of the concentration profile. The initial arsenic content of the soil was in the range of 195.6 to 222.8  $\mu\text{g/g}$  for columns A and B and in the range of 170.4 to 183.9  $\mu\text{g/g}$  for column C. The above ranges were established by analyzing 10 soil samples using SDDC method (section 3.2.2).

The total amount of arsenic leached in ~ 59 hours was ~ 12% of the total initial arsenic in the soil. Soil was once again analyzed for total arsenic at the end of the experimental period. Mass balance errors were calculated by comparing the difference between the initial and final arsenic concentration on the soil with that of the total arsenic detected in



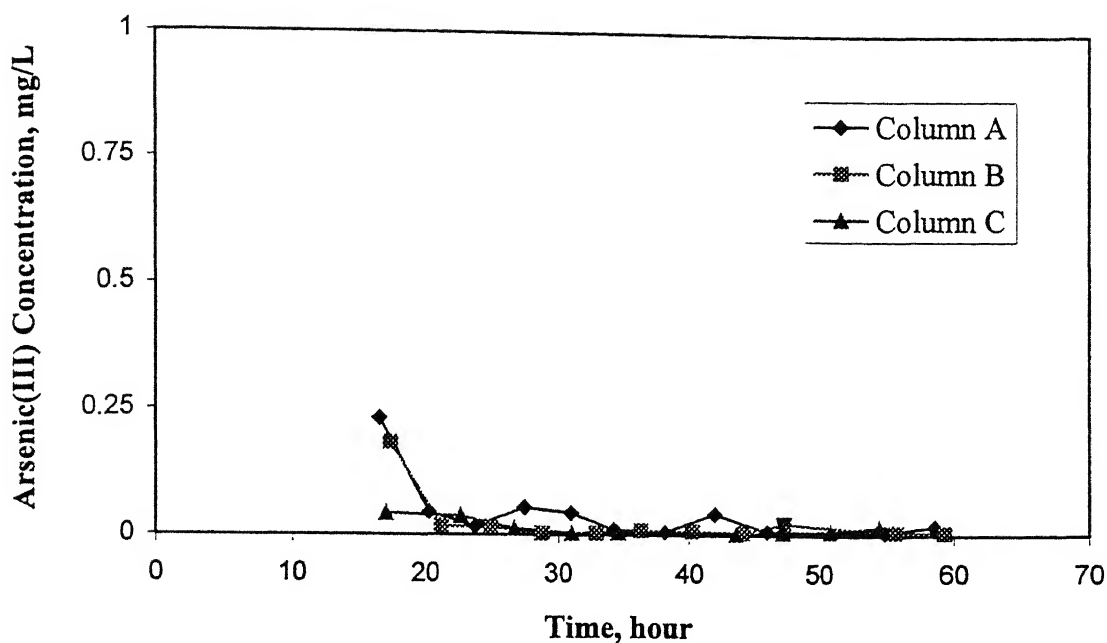


Figure 4.1 (a) Arsenic(III) leaching profile from soil with time in groundwater under aerobic condition.

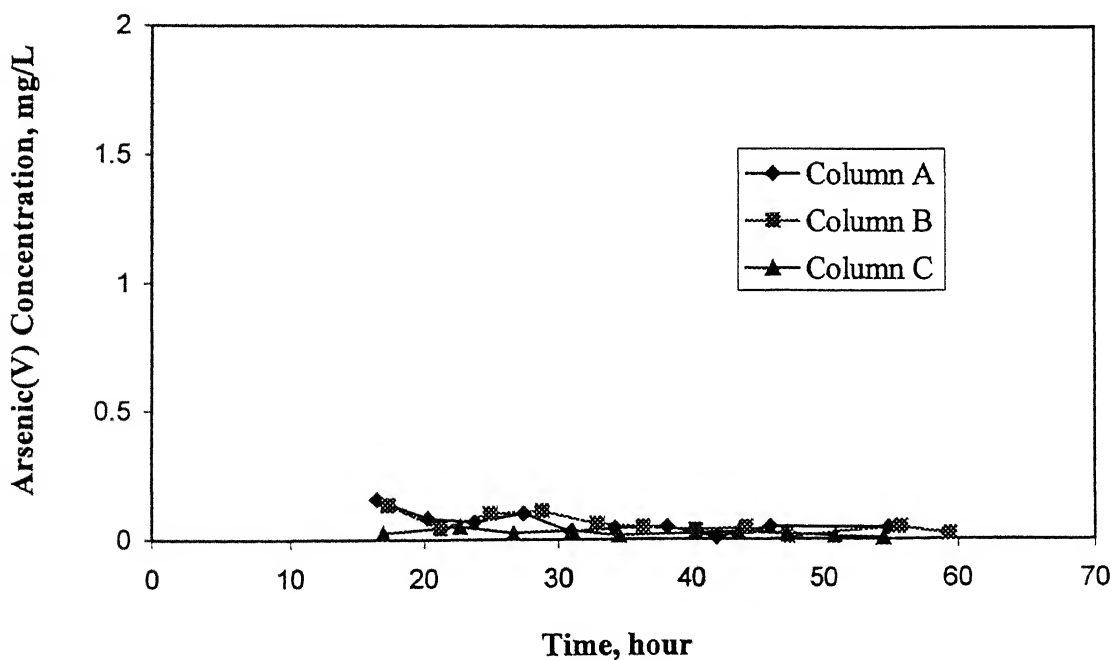


Figure 4.1 (b) Arsenic(V) leaching profile from soil with time in groundwater under aerobic condition.

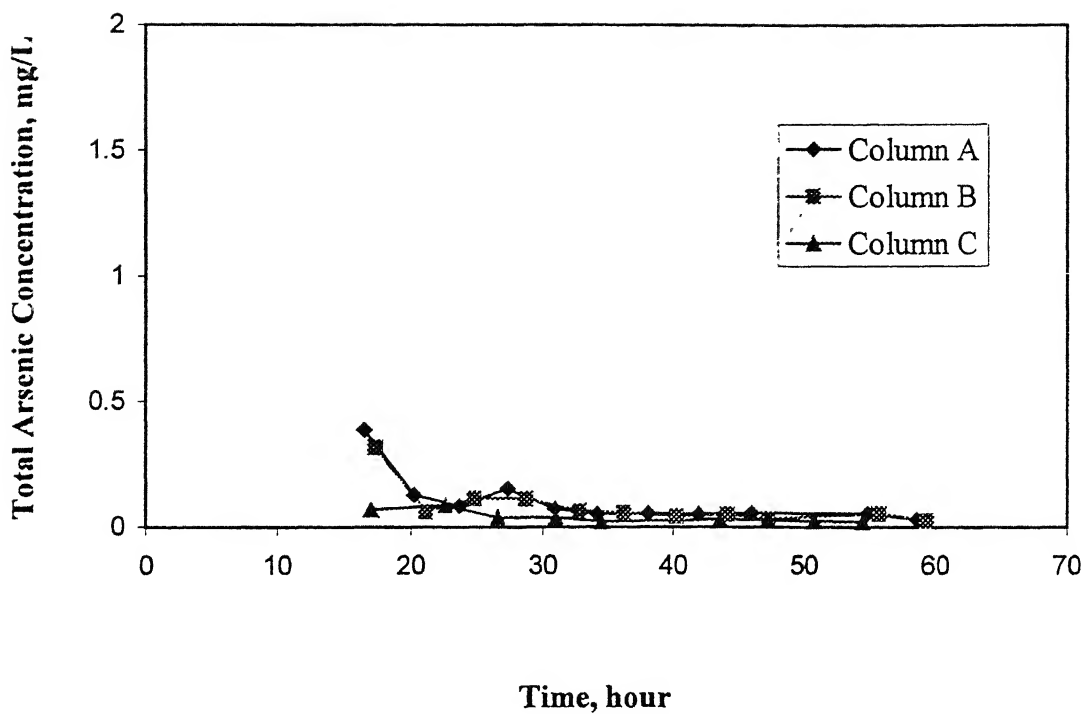


Figure 4.1 (c) Total Arsenic leaching profile from soil with time in groundwater under aerobic condition (SDDC method).

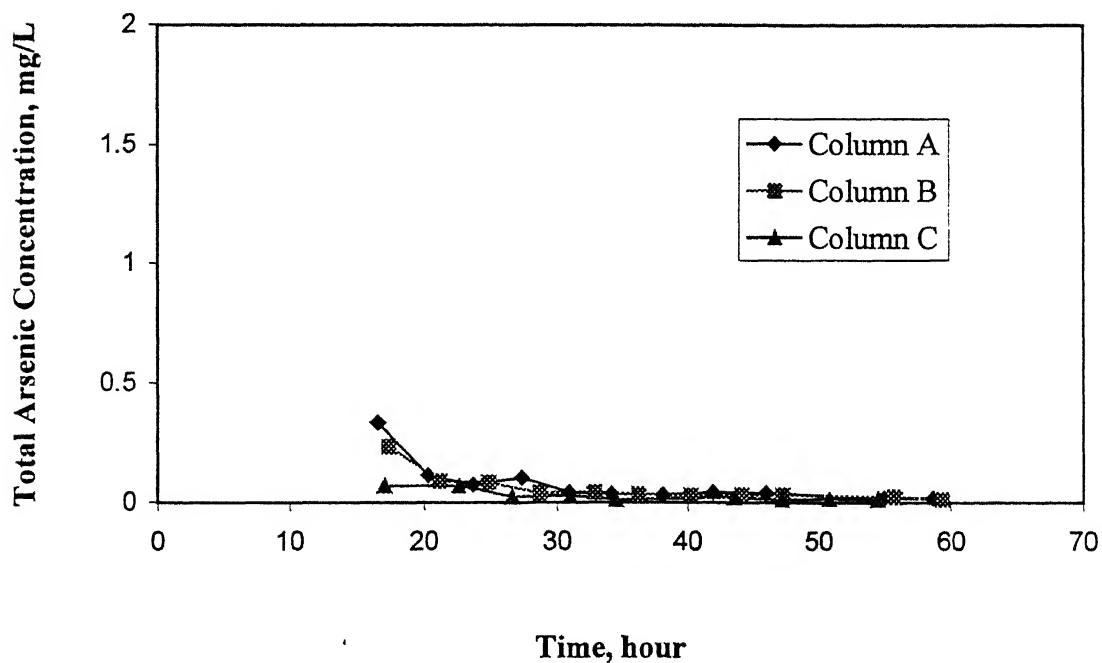


Figure 4.1 (d) Total Arsenic leaching profile from soil with time in groundwater under aerobic condition (Molybdenum Blue method).

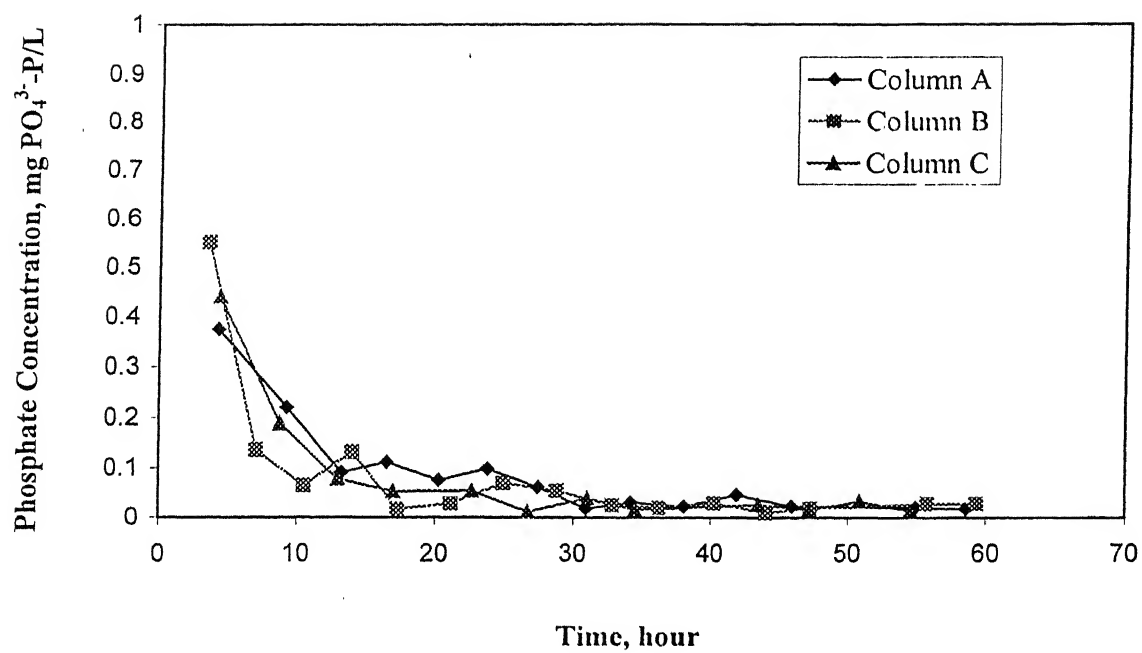


Figure 4.1(e) Variation of phosphate with time in groundwater under aerobic conditions.

the leachates. The errors were 5% and 7% for the Molybdenum Blue method and SDDC method, respectively.

It was observed that the concentration of arsenic(III), arsenic(V) and total arsenic leaching into the groundwater was very high in the initial stages (0-15 hours). As the experiments progressed, the arsenic concentrations leaching out from all the three columns reached a steady value. The initial high concentration of arsenic can be attributed to the presence of loose arsenic on the soil. Since arsenic was loaded on the soil, it is likely that some amount of loose arsenic has to be encountered with. This loose arsenic was washed away in the initial stages that gave a very high concentration of arsenic in water. This was observed not only in these experiments but in all the subsequent experiments described in the later sections. It is clear from the Figures 4.1a-d that, the effect of this initial loose arsenic diminished within the first 15 hours of the experiment and is not shown in the figures. This initial loose arsenic accounted for ~5% of the total initial arsenic on the soil, in all the cases. After this period, the arsenic in the leachate is mainly due to the adsorbed arsenic on the soil particles and is of interest to us. This experiment was conducted for a period of approximately 59 hours. The total arsenic concentration in water at the later stage (16 – 60 hours) of the experiment was found to be in the range of 0.06 to 0.09 mg/L. This value is quite high, but this can be attributed to the very high arsenic content of the soil.

The phosphate concentrations in the leachates are shown in Figure 4.1 (e). We observe that the soil from West Bengal contain some phosphate that can be leached by groundwater. After about 10 hours, the level of phosphate in the leachate falls sharply and after about 30 hours the phosphate concentration in the leachate is comparable to that of the influent groundwater. The phosphate concentration of the groundwater of IIT Kanpur was found to be in the range of 0.017 to 0.077 mg/L. The pH of groundwater was also checked during the experiment and it was found to be in the range of 7.5 to 7.9.

## 4.2 Arsenic release in groundwater by fertilizer application

The effect of fertilizer on the leaching of arsenic in the groundwater was studied both in aerobic and anaerobic environments. In the aerobic system, the fertilizer was applied intermittently whereas, in the anaerobic system a constant concentration of fertilizer was maintained. In both the cases, the amounts of fertilizer applied were the same. The different application modes were used keeping in mind the field scenario. Since, the fertilizer is applied on the surface, the top aerobic layer is exposed to higher concentration variations of the fertilizer constituents as it percolates down with the infiltrating water. As it travels deeper down into the anaerobic layer, the variations smooth out due to dispersion.

The aerobic experiment was conducted in duplicates, and the columns were designated as columns A and B. The anaerobic column was designated as C. The details of the experiments are given elsewhere (section 3.4). Results are shown in Figures 4.2a-e. . The points in the figures indicate 4-hour average concentration of arsenic and phosphate. So the line joining points is the histogram of the concentration profile. The initial arsenic content of the soil used in the columns, was in the range of 195.6 to 222.8  $\mu\text{g/g}$  (found in 10 samples).

The total amount of arsenic leached in  $\sim 57$  hours was  $\sim 14\%$  of the total initial arsenic in the soil for the aerobic experiments, and  $\sim 20\%$  in the anaerobic experiments. Soil was once again analyzed for total arsenic at the end of the experimental period. Mass balance errors were calculated by comparing the difference between the initial and final arsenic concentration on the soil with that of the total arsenic detected in the leachates. The errors were 17% and 13% for the Molybdenum Blue method and SDDC method respectively in the aerobic experiments. The same were 9% and 11% in case of the anaerobic experiments.

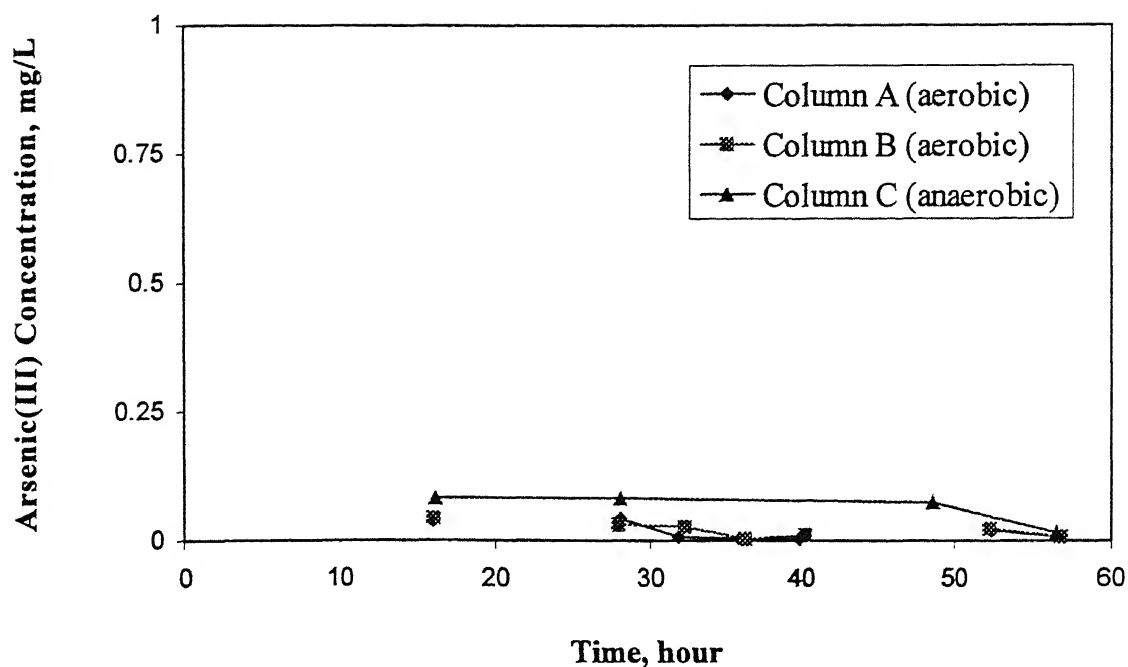


Figure 4.2 (a) Arsenic(III) leaching profile from soil with time in presence of fertilizers in aerobic and anaerobic conditions.

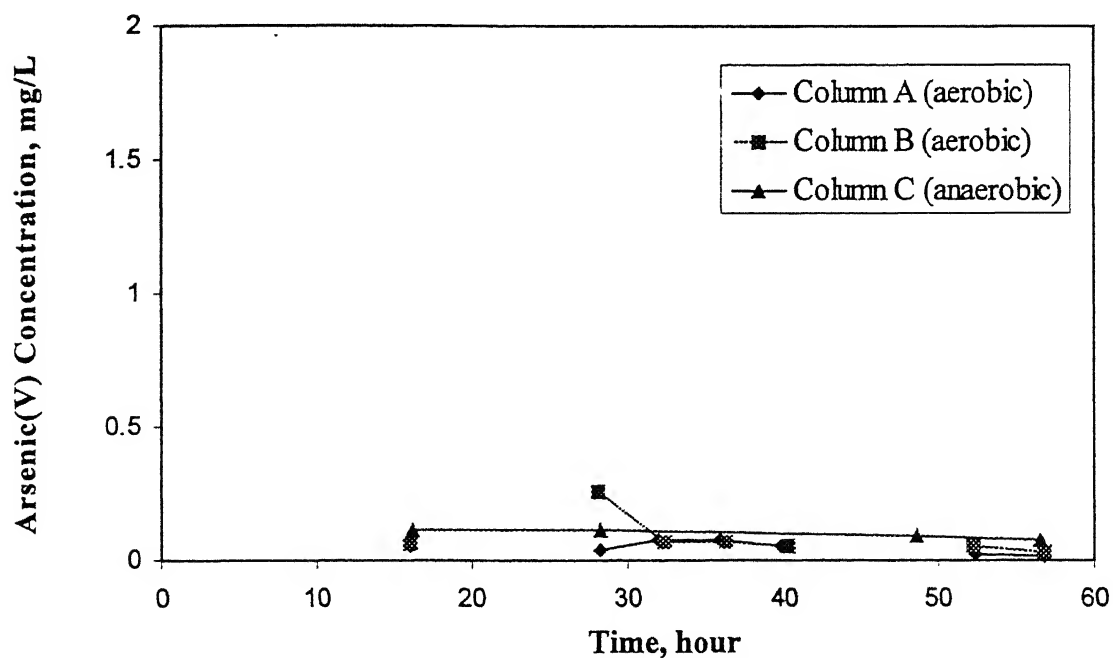


Figure 4.2 (b) Arsenic(V) leaching profile from soil with time in presence of fertilizers in aerobic and anaerobic conditions.

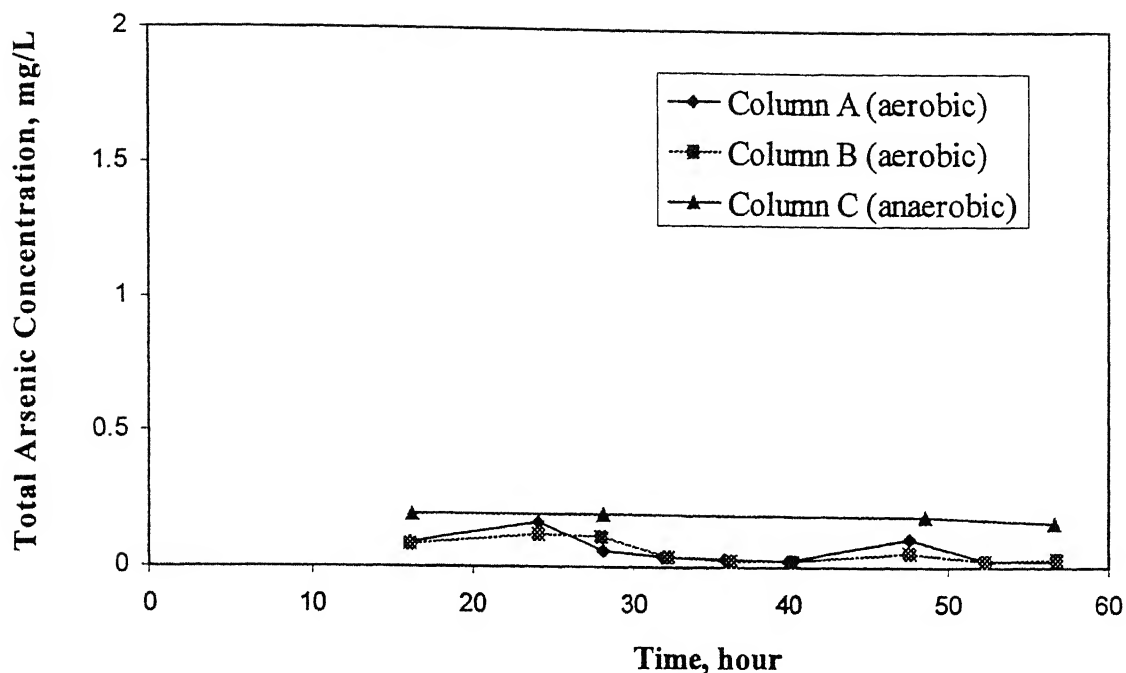


Figure 4.2 (c) Total Arsenic leaching profile from soil with time in presence of fertilizers in aerobic and anaerobic conditions as determined by the SDDC method.

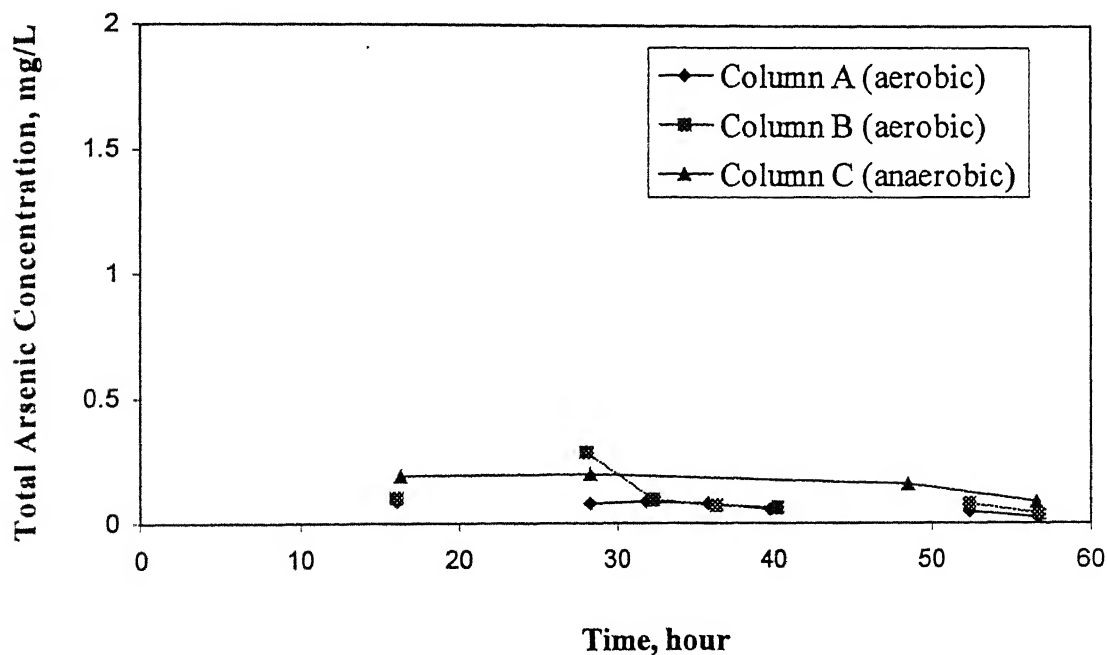


Figure 4.2 (d) Total Arsenic leaching profile from soil with time in presence of fertilizers in aerobic and anaerobic conditions as determined by the Molybdenum Blue method.

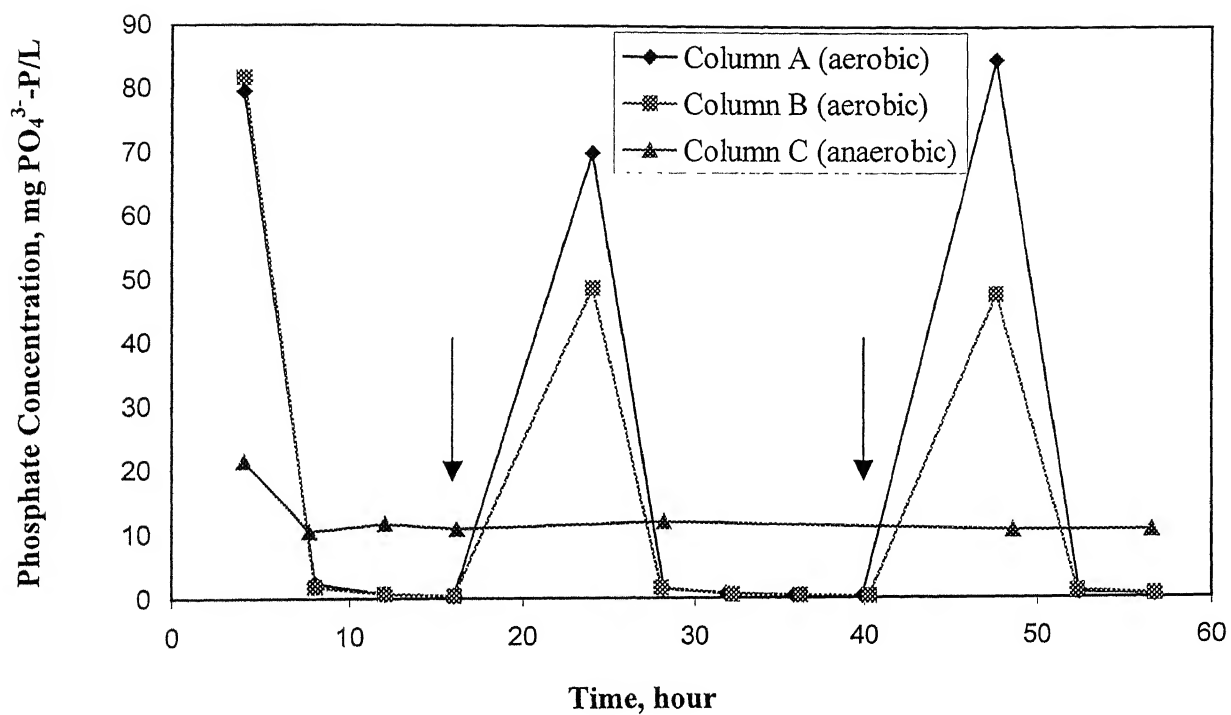


Figure 4.2 (e) Variation of Phosphate with time in groundwater with fertilizer application in both aerobic and anaerobic conditions. (Arrows in the graph indicate the points of fertilizer application in the aerobic experiments).



The SDDC method seems to be a more reliable method for determination in cases where phosphate concentrations are much higher than the arsenic concentrations because very high phosphate concentrations interfere with the determination of low arsenic concentrations in the Molybdenum Blue method. In the set of aerobic experiments, phosphate was added after collection of every ~1 L leachate. This led to sudden, appreciable increase in the phosphate concentration as is evident from Figure 4.2e, which resulted in non-detectable arsenic concentrations by the Molybdenum Blue method. As such, the Figures 4.2(a-c), all have two discontinuities present immediately after the points where phosphate was added. Although the speciation of arsenic could not be obtained at these points, the concentration of total arsenic leached out was still obtained by means of the SDDC method.

The arsenic concentration profiles in the leachates (Figures 4.1a–d) were very similar to that seen in the experiment with groundwater only. After the initial (15 hours) washout of loosely bound arsenic, the leachates in aerobic experiments contain about 0.02–0.08 mg/L of total arsenic. The anaerobic column in which a constant fertilizer concentration was applied, on the other hand, shows an appreciable increase in the arsenic concentration leaching in the groundwater, which was found to be 0.16 mg/L (averaged over the time period from 16 hours to the end of the experiment).

The majority of the groundwater where arsenic is found in the Ganges Delta aquifer has been reported to be anoxic in nature (Nickson *et al.*, 1999, 2000). The small increase in arsenic concentration under anaerobic condition indicates that, the phosphate may slightly enhance the mobilization of arsenic in the Ganges Delta Aquifer as was proposed by Acharyya *et al.*, (1999, 2000). However, in the aerobic experiments, no appreciable increase in the total arsenic concentration was obtained corresponding to the increase in the phosphate concentration.

One difference between the experiments conducted here and the field scenario is the time of contact between the water and the soil. Typical flow velocities maintained in these column experiments were approximately in the range of 17 to 25 cm/hr, as compared to that of 500 cm/year (Mallick and Rajagopal, 1996) in the West Bengal aquifer. On the other hand natural soil arsenic contamination is aged as compared to fresh arsenic loaded soil used in this study. The results indicate that the effect of phosphate in physico-chemical leaching of arsenic from the soil may not contribute significantly to the arsenic contamination in water.

### **4.3 Arsenic Release in Groundwater Mediated by Iron-reducing Bacteria**

This experiment was conducted to study the role of iron reducing bacteria in the release of arsenic in the groundwater of IIT Kanpur under flow conditions through a fixed bed. The details of this experiment are given elsewhere (section 3.4.2). Results are shown in Figures 4.3a-e. The points in the figures indicate 4-hour average concentration of arsenic and phosphate. So the line joining points is the histogram of the concentration profile. The initial arsenic content of the soil used in the column, are in the range 195.6 to 222.8  $\mu\text{g/g}$  of soil (found in 10 samples).

The total amount of arsenic leached in ~118 hours was ~53% of the total initial arsenic in the soil. The amount released after ~50 and ~66 hours are ~27% and ~34%, respectively. Soil was once again analyzed for total arsenic at the end of the experimental period. Mass balance errors were calculated by comparing the difference between the initial and final arsenic concentration on the soil with that of the total arsenic detected in the leachates. The errors were 8% and 7% for the Molybdenum Blue method and SDDC method, respectively.

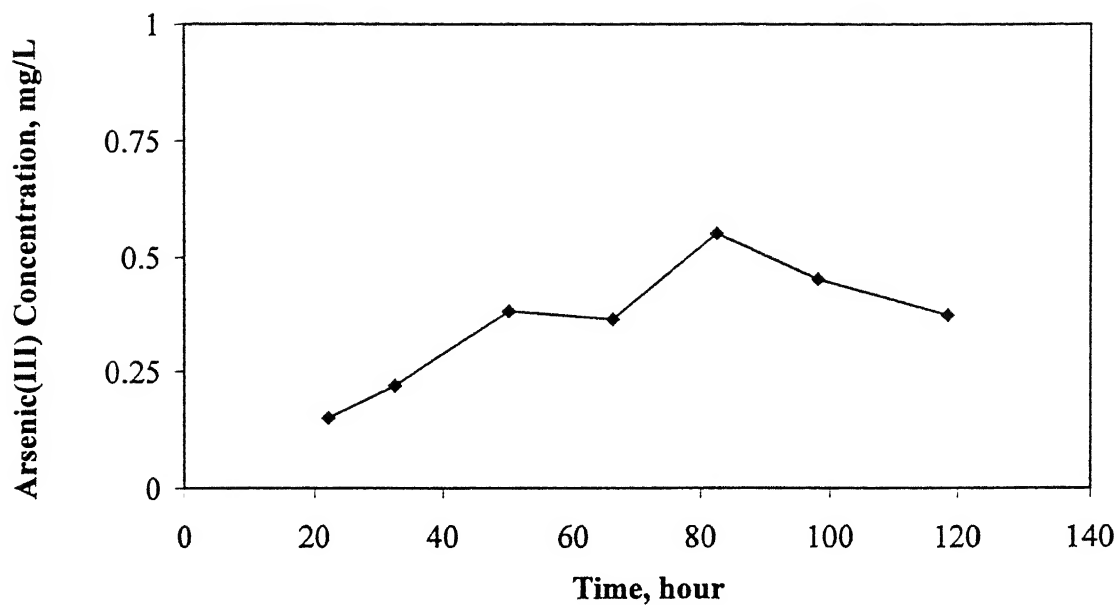


Figure 4.3 (a) Arsenic(III) leaching profile from soil with time in presence of iron-reducing bacteria.

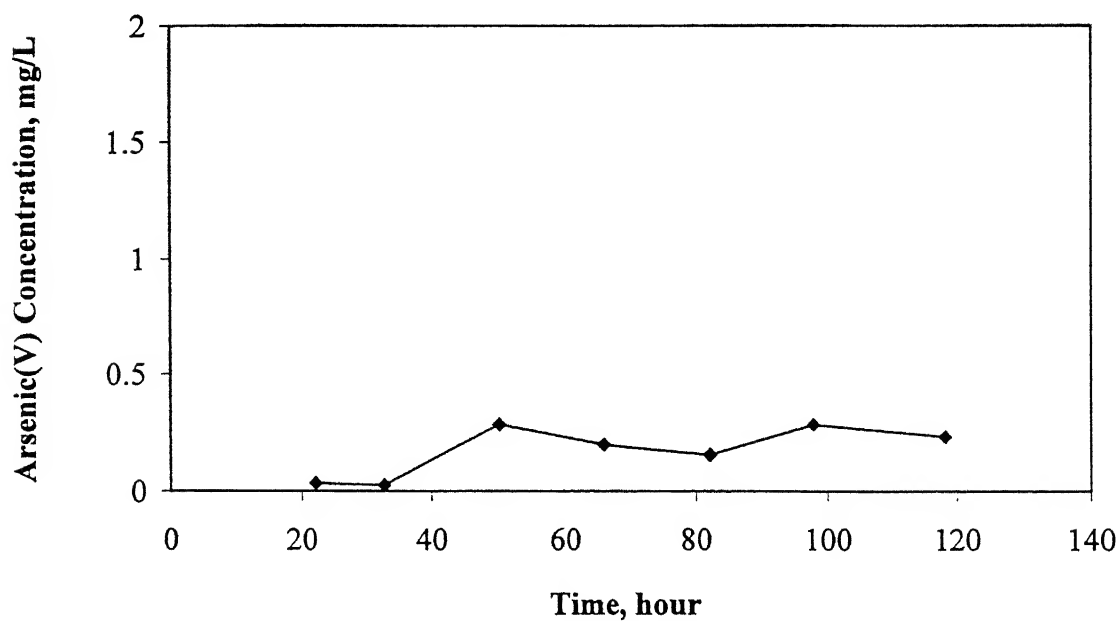


Figure 4.3 (b) Arsenic(V) leaching profile from soil with time in presence of iron-reducing bacteria.

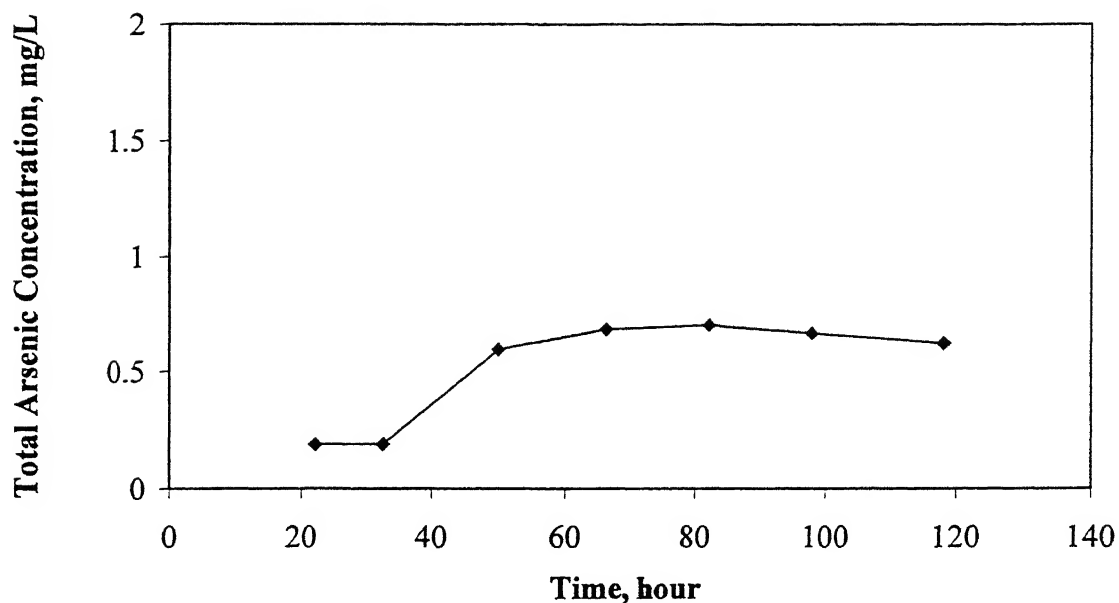


Figure 4.3 (c) Total Arsenic leaching profile from soil with time in presence of iron-reducing bacteria (SDDC method).

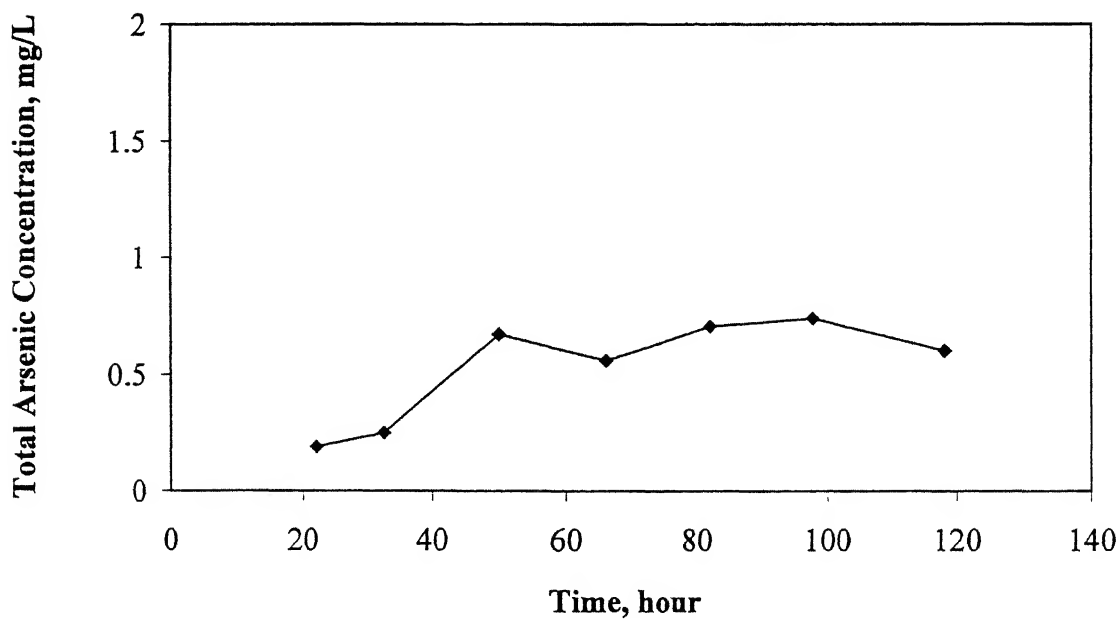


Figure 4.3 (d) Total Arsenic leaching profile from soil with time in presence of iron-reducing bacteria (Molybdenum Blue method).

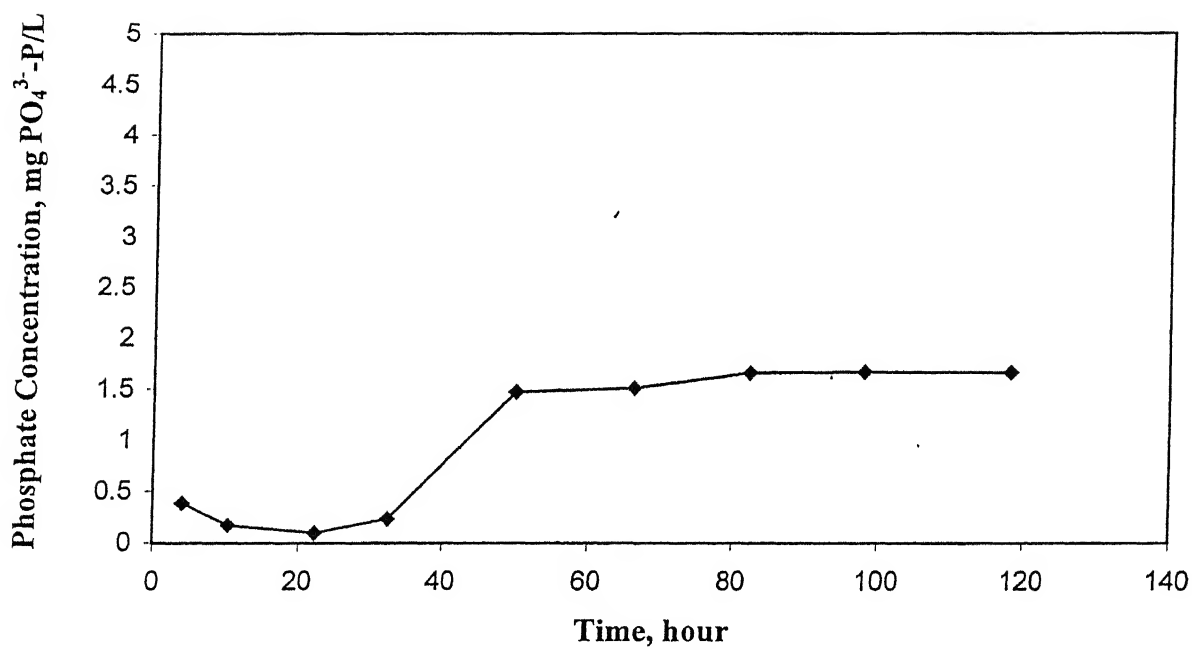


Figure 4.3 (c) Variation of phosphate concentration with time in case of bacteria in groundwater under anaerobic conditions.

In this case also, the initial loose arsenic effect was observed, resulting in very high arsenic concentrations in the water in the first 15 hours. The decreasing trend in the concentration extends up to 20 hours. The concentration started increasing beyond 20 hours and at the end of 30 hours, a marked increase in the effluent arsenic concentration was detected which continued for the rest of the duration of the experiment. The later increase in the arsenic concentration was accompanied by an increase in the phosphate concentration. Influent water contained some phosphate as part of the bacterial culture solution. The time lag for the influent phosphate to appear in the effluent was about 1 hour as was observed in the previous experiments with groundwater and phosphate, and was also observed in the experiment described in the later section. So, this increase of phosphate concentration at a lag of 30 hours cannot be attributed to the phosphate in the influent water. The arsenic release due to phosphate is also not significant ( $<0.16$  mg/L) as was seen in the earlier experiment. So, the increase in the arsenic concentration cannot be attributed to the increase in the phosphate concentration. The time lag between the enhancement of arsenic release between 10 and 30 hours may be attributed to the lag time for the bacterial activity. It appears that as the microbiological activity starts in the column, it mobilizes the arsenic as well as phosphate from the soil surface. As a result we observe corresponding increases in the phosphate and arsenic concentrations in the effluent water. The total arsenic concentration in the effluent water was 0.52 mg/L as compared to 0.06 - 0.16 mg/L found in the abiotic experiments. This indicates that the iron reducing bacteria can increase the mobilization of arsenic from the soil surface to a large extent.

#### **4.4 Effect of fertilizer on the Release of Arsenic in Groundwater Mediated by Iron-reducing Bacteria**

These experiments were conducted to study the effect of fertilizer on the iron-reducing bacteria mediated release of arsenic in the groundwater of IIT Kanpur under flow conditions through a fixed bed. The details of this experiment are given elsewhere (section 3.4.2). Results are shown in Figures 4.4a-e. The points in the figures indicate 4-hour average concentration of arsenic and phosphate. So the line joining points is the histogram of the concentration profile. The initial arsenic content of the soil used in the column, are in the range 195.6 to 222.8  $\mu\text{g/g}$  of soil (found in 10 samples).

The total amount of arsenic leached in 143 hours was 82% of the total initial arsenic in the soil. The amount that is leached in 60 hours is ~53%. Soil was once again analyzed for total arsenic at the end of the experimental period. Mass balance errors were calculated by comparing the difference between the initial and final arsenic concentration on the soil with that of the total arsenic detected in the leachates. The errors were 3.8% and 5.1% for the Molybdenum Blue method and SDDC method respectively.

In this case also, the initial loose arsenic effect was observed, resulting in very high arsenic concentrations in the water in the first 16 hours. After that the arsenic concentrations leaching out remain at approximately constant (average value 0.92 mg/L), up to 104 hours. Beyond this point, the curves show a downward trend, indicating a decrease in the concentration of arsenic leaching with further progress in the experiment. However, by this time about 75% of the total arsenic content of the soil was already leached out and the decrease in concentration may be due to the deficiency in arsenic content of the soil in the latter stages of the experiment. The phosphate concentrations in

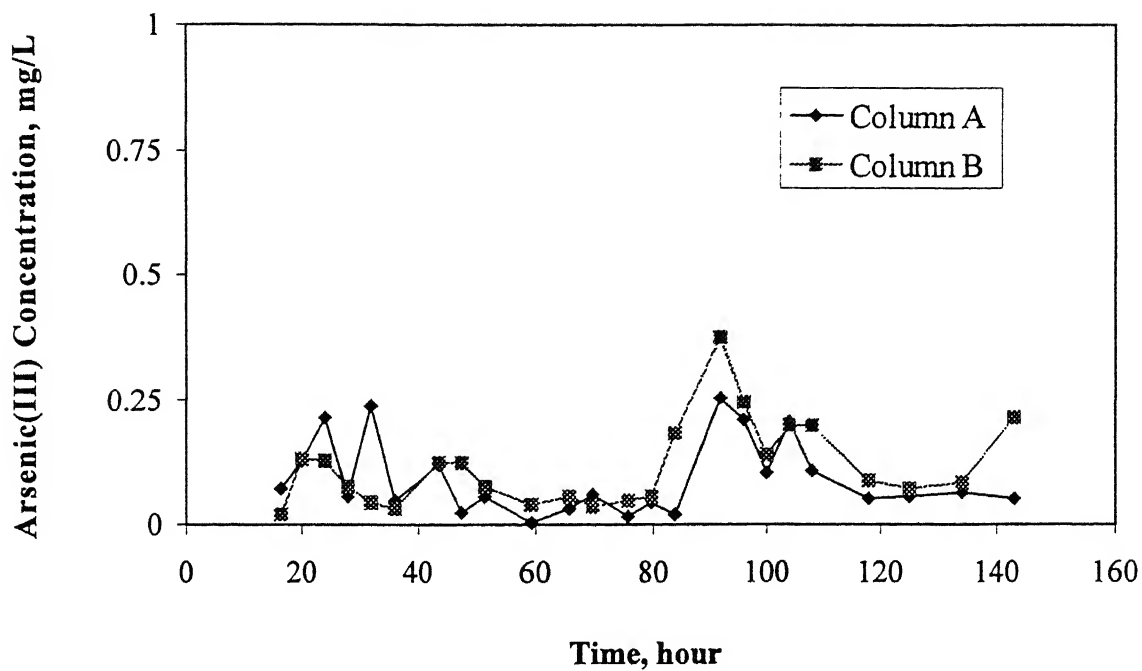


Figure 4.4 (a) Arsenic(III) leaching profile from soil with time in presence of iron-reducing bacteria and fertilizer.

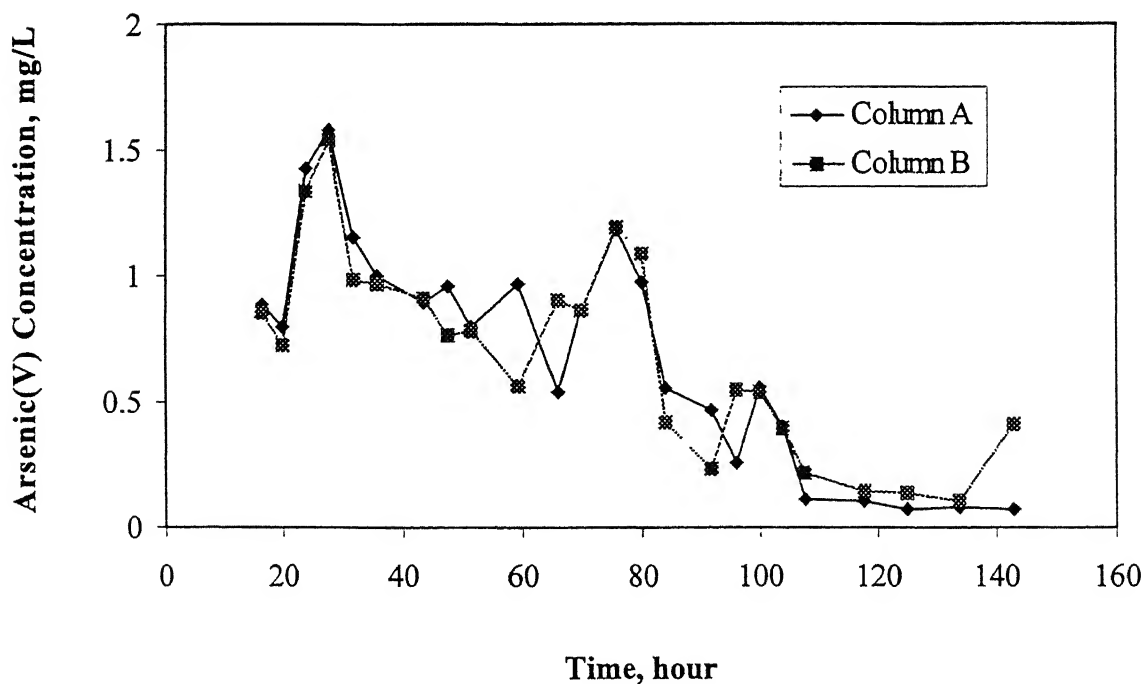


Figure 4.4 (b) Arsenic(V) leaching profile from soil with time in presence of iron-reducing bacteria and fertilizer.



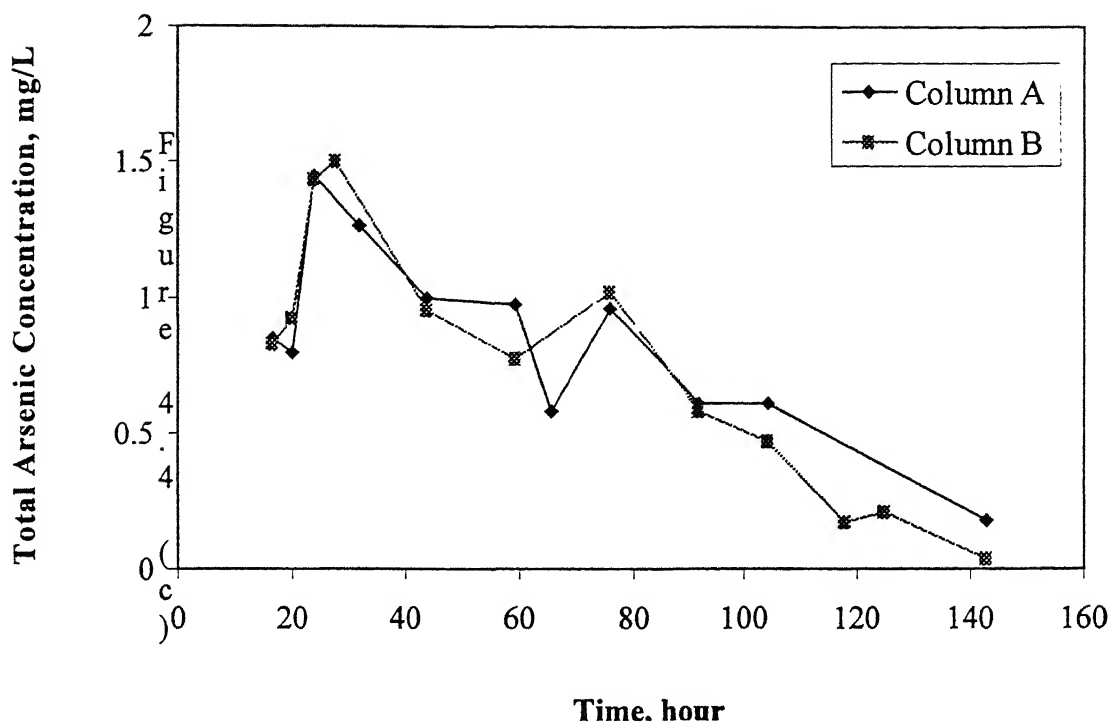


Figure 4.4 (c) Total Arsenic leaching profile from soil with time in presence of iron-reducing bacteria and fertilizer (SDDC Method).

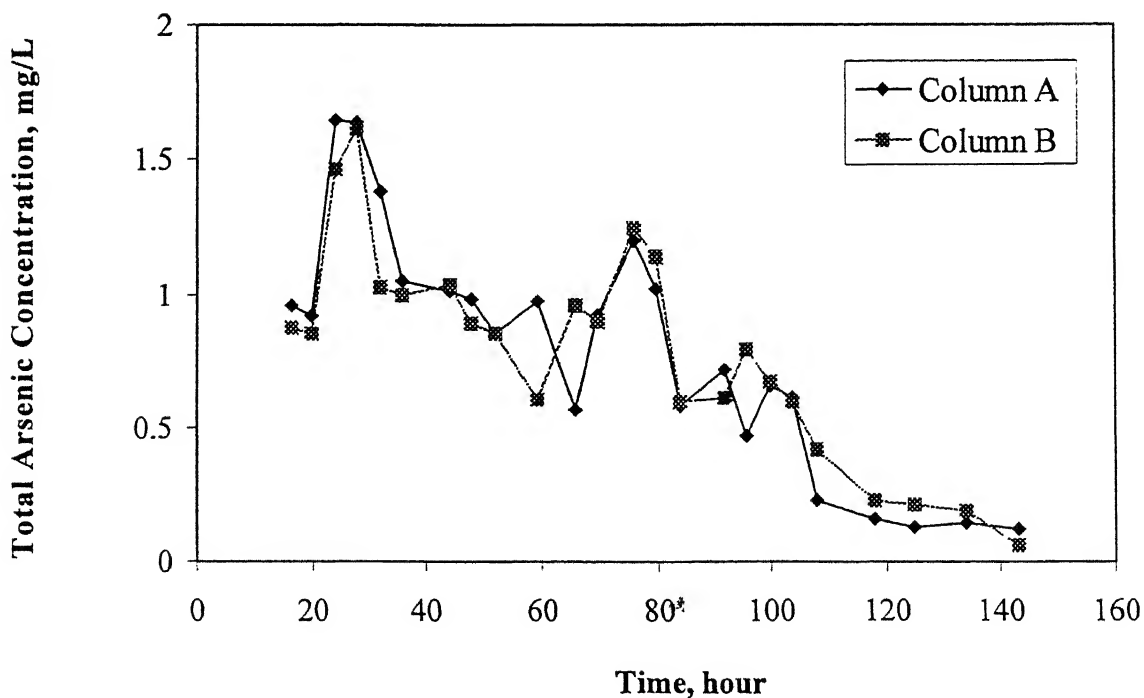


Figure 4.4 (d) Total Arsenic leaching profile from soil with time in presence of iron-reducing bacteria and fertilizer (Molybdenum Blue method).

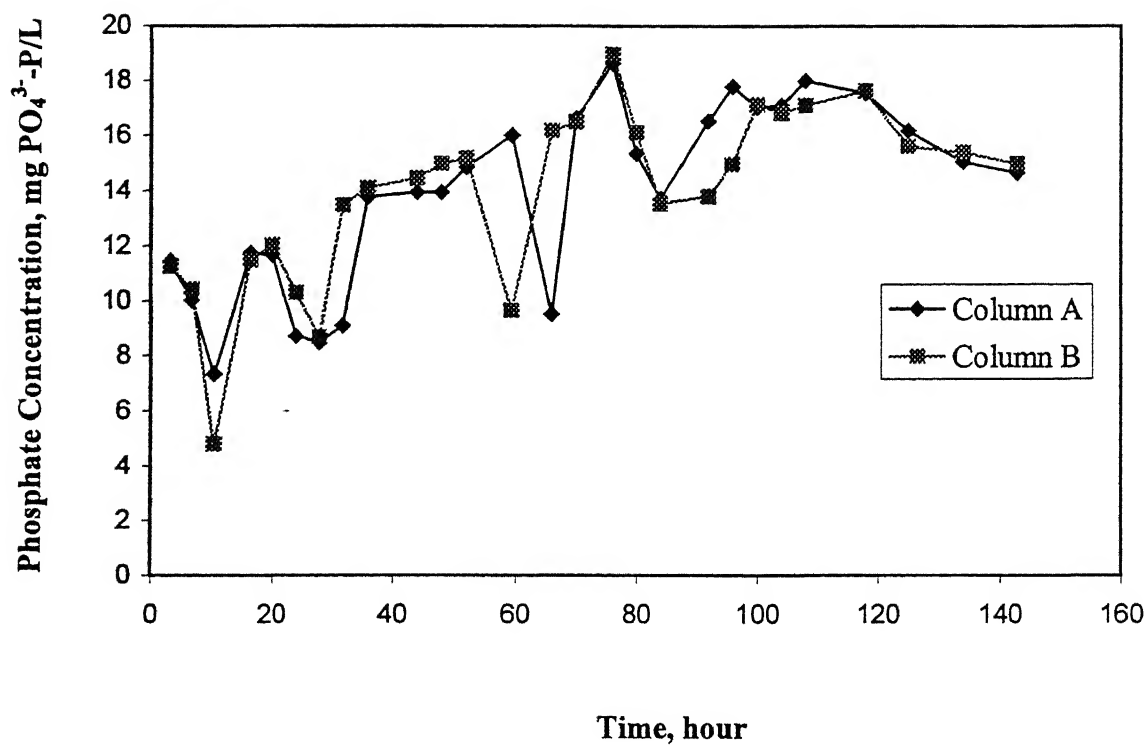


Figure 4.4 (e) Variation of phosphate concentration with time in case of bacteria and fertilizer in groundwater under anaerobic conditions.

the leachate were high from the beginning of the experiment. This is obviously due to the fertilizer added.

#### **4.5 Discussion of the Observed Results**

All the above experiments had one thing in common, and that is very high arsenic concentrations in the initial stages of the experiments. As arsenic was loaded on to the soil particles, this effect can be attributed to the loose arsenic associated with the soil. All the experiments had this effect of loose arsenic in the initial stages (first 10-15 hours), after which the arsenic concentration reached a steady value. In all the cases, the total loose arsenic accounted for only ~5% of the total initial arsenic on the soil. Thus, while comparing the various observations that were obtained from all the experiments performed, the first 15 hours were not considered.

The comparative results of all the experiments are shown in Figures 4.5a,b. The points in the figures indicate 4-hour average concentration of arsenic and phosphate. So the line joining points is the histogram of the concentration profile. The area under the curve that indicates the total amount of arsenic leached, is clearly several times higher in the cases where bacteria is present compared to the abiotic cases. The maximum amount of arsenic leached in the presence of bacteria was 82% of the total initial arsenic when fertilizer was added and 53% in the absence of fertilizer. In the abiotic experiments, maximum amount of arsenic leached was only 20% when fertilizer was added under anaerobic condition. In ~60 hours (the time for which the aerobic columns were run), the amount of arsenic released by bacteria in presence of fertilizer is ~53%, and in absence of fertilizers it is ~30%. The average concentrations in the leachates were 0.92 mg/L in the presence of bacteria and fertilizer, and 0.52 mg/L in the presence of bacteria but without any addition of fertilizer. Whereas the maximum concentration of arsenic in the leachates of the abiotic experiments never exceeded 0.2 mg/L. This clearly shows

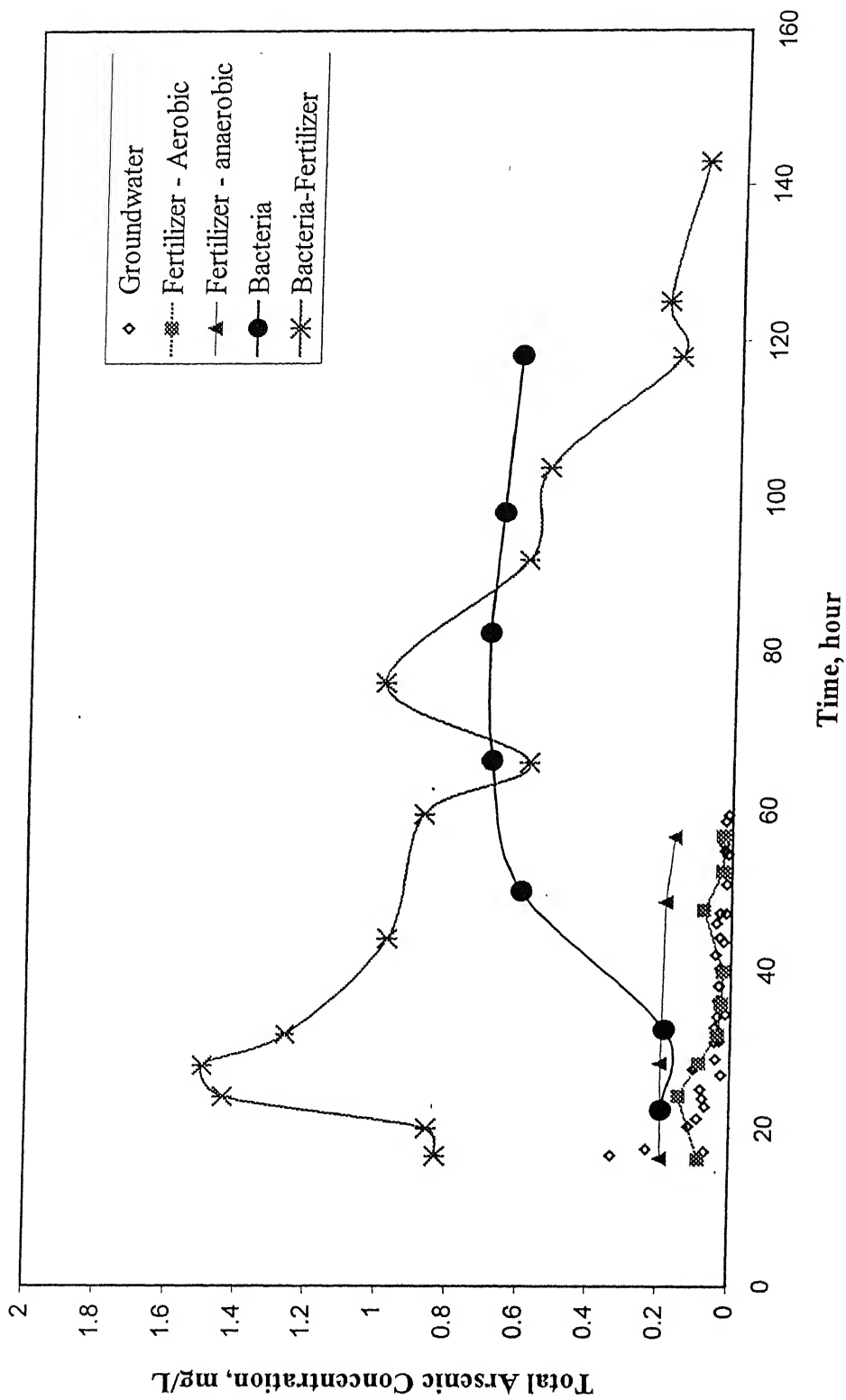


Figure 4.5 (a) Comparative study of the total amount of arsenic concentrations leached from 16 hours to the end in all the experiments as determined by the SDDC method.

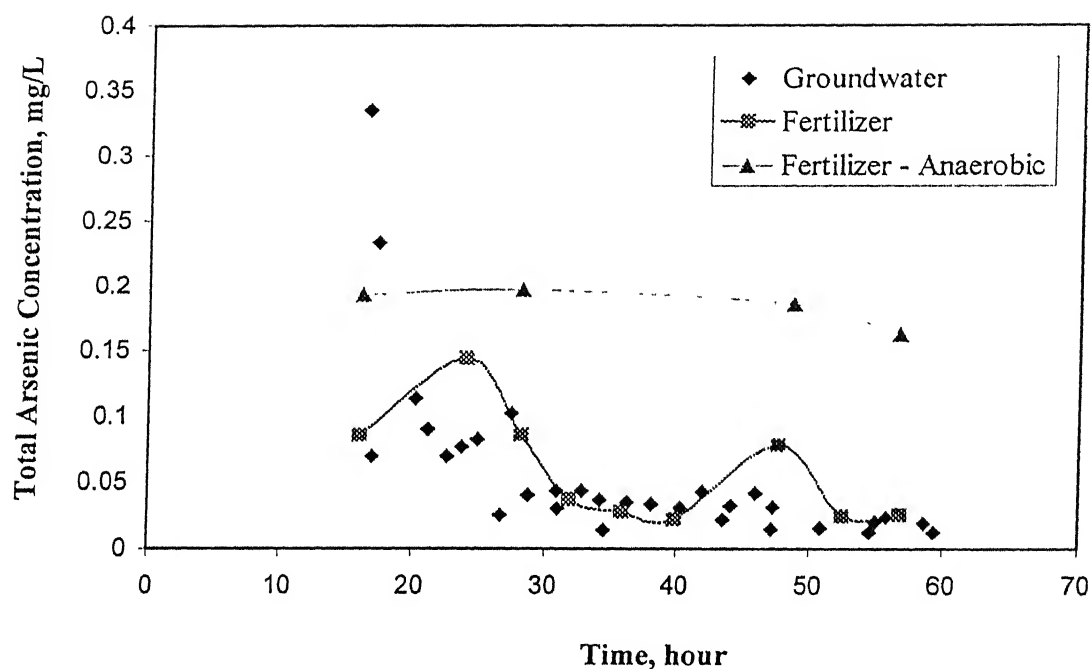


Figure 4.5 (b) Comparative study of the total arsenic leaching out in groundwater in the abiotic experiments from 16 hours to the end of the experiments as determined by the SDDC method.

that the iron reducing bacteria can mobilize significantly higher amounts of arsenic from the soil and more so if fertilizers from the top layer reaches the biotically active layer containing arsenic.

As indicated earlier, Acharyya et al. (1999, 2000) suggested that arsenic might be mobilized physico-chemically, by phosphate. The results of the abiotic experiments are shown once again in Figure 4.5b in an enhanced scale. In this figure, the initial 15 hours results are eliminated. In the aerobic environment, groundwater resulted in elution of 12% of the total arsenic. When fertilizer was added, the total elution was 14% in the aerobic environment and 20% in the anaerobic environment. This cannot be said to be significant given that the mass balance error was about 10%. However, when we look at the concentration profile of the leachate (Figure 4.5b) of the aerobic experiment with fertilizer, we see two small peaks in the concentration profile, which corresponds to the peaks in the phosphate profile (Figure 4.2e). Similarly, the arsenic concentration in the leachate of the anaerobic experiment with fertilizer was consistently higher ( $\sim 0.18$  mg/L) than that with only groundwater (0.05 mg/L). This may indicate some effect of the phosphate but nothing conclusive can be said owing to the mass balance error. The effect, if there is any, is significantly less than the effect of iron reducing bacteria.

Mallick and Rajagopal (1986) proposed that, in the aerobic environment arsenic could be leached from the soil into the groundwater around the cone of depression of the well. The column experiments conducted in the aerobic unsaturated environment, which represents the cone of depression well, leached only 12% of the total arsenic that includes the initial loose arsenic. The maximum arsenic concentration in the leachate after 15 hours never exceeded 0.1 mg/L. One should note, that the soil used in these experiments were freshly loaded with arsenic and the concentration of arsenic on the soil was 2-4 times higher than found in the Ganges delta (Ghosh, 2000). The desorption equilibrium guides that with lower concentration of arsenic on the soil, the concentration

in the water will also be lower. The aged contamination is also likely to have kinetic limitation in the desorption. These may indicate that the aeration of the arsenic-containing layer due to widespread tube wells may not be a significant mechanism for the large-scale occurrences of arsenic at high concentrations. The absence of arsenopyrite layer in the aquifer (Acharyya et al. 199, 2000) and reports of correlation of the occurrence of arsenic with anoxic environment (Nickson et al., 1998) are also evidences to the contrary of the hypothesis proposed by Mallick and Rajagopal (1986).

# CHAPTER 5

## SUMMARY AND CONCLUSIONS

This study seems to indicate that the release of arsenic from the soil is largely influence by the microbiological processes and may be to some extent by the physico-chemical processes. The key conclusions from the study are:

- (i) iron-reducing bacteria can enhance the release of arsenic from the soil to a large extent.
- (ii) addition of fertilizer can enhance the leaching of arsenic by the bacteria.
- (iii) the physico-chemical enhancement of arsenic release by phosphate is arguable and less significant than the microbiological enhancement.
- (iv) the arsenic release under the aerobic condition is not likely to be a significant mechanism in the Ganges delta.



# CHAPTER 6

## SCOPE OF FUTURE WORK

Based on the results of the study carried out it is clear that more work is needed to understand the mechanism of leaching of arsenic from the sediments in the groundwater of the Ganges delta. The following recommendations can be made for future research:

- Sand used in the columns was loaded with arsenic. Work has to be done with contaminated soil samples from the West Bengal aquifer to validate these results.
- Field experiments with depth specific sampling have to be carried out to show the effect of bacteria and fertilizer on the release of arsenic.

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# APPENDIX

## The Molybdenum Blue Method

Three 40 mL portion of the samples were taken in three 100 mL Erlenmeyer flasks, and marked as “O”, “U” and “R” to indicate the oxidized, untreated and reduced aliquots. To the oxidized aliquot 1 mL of 1 N hydrochloric acid (HCl) was added followed by two drops of 50% saturated potassium iodate ( $\text{KIO}_3$ ) solution and kept for fifteen minutes for complete oxidation of As(III) to As(V). Four millilitres of Mixed Reagent (Murphy and Riley, 1962) were added to both the oxidized and untreated aliquots, and mixed immediately. Two drops of potassium iodate ( $\text{KIO}_3$ ) solution followed by 1 mL of 1 N HCl was then added to the untreated aliquots, and mixed well. These were then kept in a water bath maintained at a constant temperature of  $40^\circ\text{C}$  for a period of 2 hours.

To the reduced aliquots 4 mL of Reducing Reagent (Johnson and Pilson, 1972) was added, mixed well and allowed to react for 3 hours. Then 4 mL of mixed reagent was added and 15 minutes given for colour development.

The absorbances of all the samples were measured at 865 nm using a path-length of either 10 mm or 40 mm.

- Mixed reagent (Murphy and Riley, 1962): 125 mL of a 5 N sulphuric acid ( $\text{H}_2\text{SO}_4$ ) solution and 37.5 mL of a 0.032 M ammonium molybdate [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ] solution were mixed thoroughly. Then, 75 mL of 0.1 M ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ) and 12.5 mL of a 0.0082 M potassium antimonyl tartrate [ $\text{K}(\text{SbO})_4\text{H}_4\text{O}_6\cdot 4\text{H}_2\text{O}$ ] solution were added successively with through mixing after each addition. This reagent was to be prepared fresh when required.

Reducing reagent: 20 mL of a 3.5 N sulphuric acid ( $\text{H}_2\text{SO}_4$ ) solution was added to 40 mL of a 0.74 M sodium metabisulfide ( $\text{Na}_2\text{S}_2\text{O}_5$ ) solution and mixed thoroughly. Finally, 40 mL of a 0.056 M sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_5$ ) solution were added to it with through mixing. If refrigerated, the reducing reagent is stable for up to 24 hrs.